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Permanent Vein Cannulation for Repeated Hemodialysis

By

S. Giovannetti, A. Bigalli, L. Cioni, M. Della Santa and P L. Balestri

This important practical problem, first tackled in 1951 by Alwall (1) was solved in 1960 by Quinton et al. (8) The cannulation technic used by these authors consists in the placement of a Teflon indwelling cannula into the radial *artery near the wrist joint, and of an*other cannula into a suitable vein of the same forearm the outer extremities of the two cannulas are then connected and a permanent arterio-venous shunt is thus produced When dialysis is wanted the connection is removed and the two cannulas are connected to the dialyzer

This technic was tried by several authors and the results were favorable on the whole, with some exceptions which will be discussed later on. We too tried this procedure on three patients and observed no severe complications but clotting in one case.

However the disadvantages inherent in the execution of dialysis with the artery to vein system (i.e. hyperdynamic circulation, low availability of blood, etc.) induced us to try permanent cannulation of the veins so that many dialyses could be performed with the system vein to vein.

In the present paper this attempt is described and the results reported.

Material and methods

The Teflon cannulas were supplied to us by Tally Reg. Co[1] the sizes employed being those indicated by Quinton et al. (8) for the fore arm bypass, 1/8 inch. Sometimes, when the large dimensions of the veins suggested it, larger size cannulas were employed.

Extracorporeal hemodialyses were performed by means of an artificial kidney previously described when it still was under experiment (3). This apparatus was improved in several respects as will be reported elsewhere.

A Sigmamotor pump (Mod. T.6.S.) was always used for the blood circulation in the dialyzator and no hemolysis was observed not even when dialysis was prolonged as long as 12 hours. Polyvinyl tubing and siliconized glass connections were used to attach the patient to the artifical kidney

[1] Tally Register Corporation, 1310 Mercer Street, Seattle 8 Washington, U.S.A.

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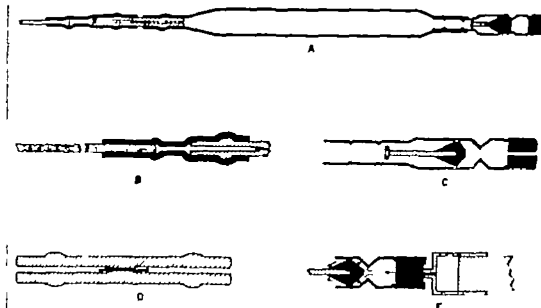


Fig 1 A schematic representation of the apparatus for prolonged cannulation of the veins. The apparatus completely assembled (A) the Teflon cannula for the safena vein and the glass capillary connected to it by the rubber tube (B) the filling valve opened (C) the glass capillary (D) the valve being filled with a syringe (E) The glass pieces of the apparatus are shaded, the rubber pieces are black and the Teflon cannulas are dotted.

Technic for cannulation of the veins

Each vein is cannulated with a Teflon cannula which is connected with the apparatus for the slow and continuous infusion of a dilute heparin solution (fig 1)

The cannula to be used for the safena vein is 30—35 cm long and is perforated near its tip with many holes with smooth borders, 1 mm in diameter (fig 1B) which prevent the obstruction of the cannula itself, when suction is made by the Sigmamotor pump Th Teflon cannula to be introduced into an arm vein is 20—25 cm long and bears no lateral holes. In both the safena and the arm vein cannulas a step is moulded at the point of their shaft where they will emerge from the skin (see below)

These Teflon cannulas are connected with the apparatus for the slow infusion by a rubber tube which is long enough to prevent the occasional movements of the apparatus being transmitted to the Teflon catheter itself The lumen of this rubber tube is narrower than the outer diameter of the Teflon cannulas and its walls are sufficiently thick to ensure good connection with the cannula at one end and with the slow infusion apparatus at the other end (fig 1B)

The apparatus for the continuous slow infusion is composed of a capillary glass cannula (fig 1D) of a rubber container (fig 1A) and of a valve (fig 1C) for filling it by means of a syringe (fig 1E)

The glass capillary is prepared from a tube 5—7 cm long having a lumen diameter of about 1 mm and an outer diameter of 6.0—6 5 mm The central portion of this tube is narrowed in a flame, an amianthus thread (1 0 cm long) having been introduced into it. This amianthus thread prevents the glass walls of the tube sealing together when heated and pulled out and preserves the capillary lumen Every cannula is tested as to the flow rate it allows when completely assembled to the apparatus whose container has been filled, and those cannulas whose flow rate is not within the range of 1 0 and 2.0 ml/hour are discarded This preliminary test discloses the emptying time of the apparatus, thus indicating the time intervals and the fluid volume which are necessary to ensure a continuous flow of the dilute heparin solution.

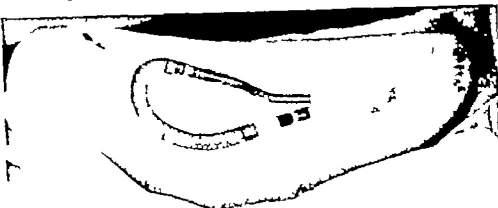


Fig. 2. An apparatus for prolonged cannulation of the veins in place in the safena since 9 days. This apparatus functioned perfectly for 32 days and 6 dialyses were performed with it (case 2).

I may be noted in this connection, that the continuous functioning of the cannulas for many days makes their flow rate more and more slow It is then necessary to change the apparatus with another one which has recently been checked.

The filling is generally performed every 12—24 hours with 20—30 ml of isotonic saline solution containing 60 mg of heparin and 100,000 I. U. of penicillin per 100 ml.

For dialysis the rubber connectors are clamped with hemostat, near the cannulas, the infusion pparatus is removed and the safena cannula is connected with the suction tubing of the Sigmamotor pump while the arm cannula is connected with the tubing coming from the dialyzer A preliminary check of the function of the two cannulas is advisable, which may be done by suction with syringe in the safena cannula and by infusing sterile *isotonic saline into the arm cannula.*

When no longer required, the tw cannulas may be removed by simply pulling them from the emergency wound prophylactic pressure dressing, for 24 hours, has been always sufficient to oid bleeding in our patients

The cannulation of the veins is performed under local anesthesia and with sterile technic throughout After the em is exposed through transverse incision, punctura wound is made 8—10 cm distally to the incision itself, for the outlet of the cannula from the skin (fig 2 A subcutaneous tunnel is created according to Quinton et l. (8) by blunt dissection with a bent hemostat which is introduced through the incision. The cannula is passed through this tunnel and, following its introduction into the ein and checking of its function, the vein walls are ligated on it. The cannula is then filled with dilute heparin solution through the rubber connector and the slow infusion apparatus, previously filled, is connected. The incision is sutured and over it sterile dressing is applied which is secured with adhesive tape, as is also the slow infusion pparatus. In order to avoid hemorrhage in the subcutaneous tunnel the general heparinization should be delayed several days, while the regular filling of the pparatus must be done regularly

Clinical experience with the permanent vein cannulation

Our experience in this regard was made on 4 patients, three of whom were suffering from acut renal failure, respectively anuria post abortum (case 1) anuria post partum (case 2) and anuria post transfusion (case 3) The fourth patient was suffering from chronic glomerulonephritis which had again become acut and which had been diagnosed when, after 40 days of complete anuria, a renal biopsy was performed. An earlier diagnosis was not made due to the unhelpful anamnesis and to the large size of the kidneys as found t the roentgen examination.

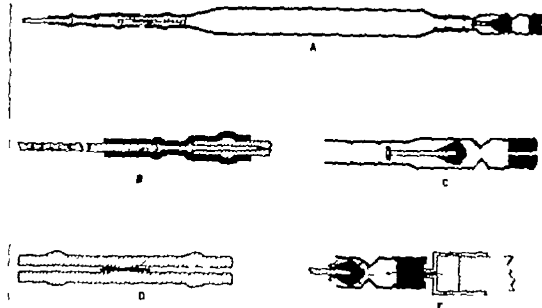


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Summary

A technic is described for prolonged cannulation of the veins for repeated hemodialysis. This technic is based on the employment of Teflon cannulas which are inserted into the safena vein and into an arm vein; the cannulas are connected with slow infusion apparatus which ensures a sluggish flow of dilute heparin solution through the cannula.

This technic, tried in four patients, appears to be completely free from dangerous complications and makes it possible to perform many hemodialyses, vein to vein, with only one preparation of the veins.

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Addendum

Since the presentation of this paper for publication three more patients have been successfully submitted to permanent cannulation of the veins. The first patient had the cannulas in place for 60 days and was submitted to 6 hemodialyses; the second for 27 days with 3 hemodialyses, and the third for 12 days with 2 hemodialyses.

No untoward effect was observed in these patients and no difficulties were met with in performing the hemodialysis.

The cannulas were removed because of recovery after 24 days in patient no. 1 and after 7 days in patient no 3 Patient no. 2 was discharged from the clinic at the insistence of her family after 37 days of complete anuria. In these three patients the cannulas were functioning during the whole period of observation and 4 dialyses were performed on patient no. 1 1 dialysis on patient no. 3 and 6 dialyses on patient no 2, without any difficulty

Patient no. 4 died as a consequence of bleeding from the wound of the renal biopsy (probably from a sclerotic artery of the kidney) after 45 days of anuria, when the safena cannula was still functioning The first arm cannula placed in this patient did not function satisfactorily after 12 days a second cannula was placed in the other arm but even this cannula failed to function after 18 days and finally a third cannula was placed which functioned until death The unsatisfactory function resulted in a high resistance to the blood flow returning to the patient during dialysis, which reached pressure values of 100—130 mm Hg with flow rates of 250—300 ml/min This was probably due to a parietal thrombosis of the vein which appeared proximally to the cannula tip, since the removed cannulas were free from clot and since it was not possible to introduce the cannulas more deeply in the vein

The cause of these thrombosis was almost certainly the infusion of a hypertonic dextrose solution in the first instance, while in the second instance we suppose that the cause was the irritating action of movements of the cannula itself on the intima of the vein

This was the only complication we encountered in the four patients studied there was never observed a symptomatology which might justify the suspicion of pulmonary embolism and no significant modification of the clotting time occurred during the intervals between dialyses.

Discussion

All the methods of permanent cannulation of the veins are doubtless effective for repeated hemodialysis in the same patient The arterio-venous bypass, according to Scribner (10) may be used when dialysis is performed with the artery to vein system and offers the peculiar advantage that it needs no supervision other than the daily cleaning of the puncture wounds on the forearm. However this method is complicated by peculiar accidents such as dangerous hemorrhages (7 9) hyperdynamic circulation (4 10) and false aneurisms (7) which make it not completely safe for the patient

The method of permanent cannulation of the veins is obviously free from those complications, but needs regular supervision of the slow infusion apparatus in order to prevent the container becoming empty and the heparin solution stopping with the resulting danger of clotting in the veins and cannulas. In our experience this complication never happened in the cannulas nor in the cannulated safena which never failed to function. Arm vein parietal thrombosis occured twice in patient no 4 probably as a consequence of our mistakes, but it cannot be excluded that such a complication in the arm vein may occur also in other patients, facilitated by the small dimensions of the cannulated vein. However this is a not dangerous complication for the patient and moreover it may be stated that occlusion of cannulas through clotting is more frequent when the arterio-venous bypass is used since it was observed by all who tried this technic (2 6 7 9) including ourselves.

As to another complication which is inherent to the methods of permanent cannulation of vessels i e. infections (7 9 11) it was never observed in our patients penicillin contained in the dilute heparin solution may have contributed to the prevention of such a complication

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Ammonia and Hepatic Coma

By

Johan Eggert

The increasing interest in the part that ammonia plays in the neuropsychiatric changes following liver insufficiency has led to this study

The origin of ammonia in the blood can be either exogenous, derived from the breakdown of nitrogen-containing substances in the gastro-intestinal tract and carried by the portal circulation to the liver or endogenous mainly formed in the liver and the kidneys.

The elimination of ammonia takes place mainly in the liver where ammonia is converted by the ornithine cycle to urea, and to some extent in the kidneys.

Both muscle and nervous tissue can also participate in the metabolism of ammonia by the reversible reactions between ammonia and α-ketoglutaric acid or glutamic acid whereby these tissues are able to remove ammonia from the blood or liberate it into the blood (17 36, 50, 52, 54 61)

A relation between ammonia and the mental and nervous disturbances in hepatic coma has been suggested for many years (8, 9 14 22, 26, 34 35) Only by the work of Davidson (38 55) McDermott (31 32) Bessman (5 6) Sherlock (44 45) and Stahl (48, 49) was a better understanding of these problems obtained.

The common conception of the etiology of hepatic coma is that ammonia formed in the gastro-intestinal tract by passes the liver through a shunt or passes unaltered through the liver on account of liver insufficiency resulting in an increased ammonium concentration in the peripheral blood This rise may affect the nervous system giving symptoms characteristic for hepatic coma.

It must be pointed out, however that other etiological possibilities exist for hepatic coma. Changes in the electrolyte balance and in carbohydrate metabolism are often found Even in the disturbed metabolism of the proteins, active substances besides ammonia are formed that act on the brain (44)

The purpose of this investigation was to estimate the clinical value of blood ammonia determination in hepatic coma and during glutamic acid therapy

Submitted for publication May 29, 1962.

Table II. Diagnosis, clinical course and treatment of patients with hepatic coma

No.	Age	Sex	Diagnosis	Precipitating factor	Treatment	Course and outcome
1	63	♂	Cirrhosis hepatic Esophageal varices Chron. pyloric ulcer	Hema-themesis	Sengstaken's tube Acromycin Glutamic acid 20 g × 2	Fall in ammonium level after glutamat N increased arterio-venous deficit. No clinical improvement. The patient died in coma
2	66	♂	Cirrhosis hepatic	Hema-themesis	Aureomycin Glutamic acid 20 g 2	On account of the low initial value only slight fall in the ammonium concentration and an insignificant increase in the arterio-venous deficit is seen after glutamate. No immediate clinical effect, but coma disappeared within 3 days. Positive ammonium tolerance test
3	78	♂	Coffey' operation Liver insufficiency?	Accumulation of urea in the bowel	Rectal tube	Fall in ammonium level and clinical improvement after treatment. Positive ammonium tolerance test
4	66	♂	Cirrhosis hepatic Chron. pyloric ulcer	Hema-themesis	Blood transfusion Enema	The patient died within 2 days under the picture of steady progression of coma
5	60	♂	Cirrhosis hepatic Gastric ulcer	Bleeding after resection of the stomach	Glutamic acid 20 g × 2	After the operation coma developed. Glutamic acid lowered the venous blood ammonia, but the arterial ammonia remained high. No clinical effect. The patient died 3 days after the outset of the coma. Positive ammonium tolerance test
6	65	♀	Diabetes mellitus Liver insufficiency?	Ammonium tolerance test	None	After an ammonium tolerance test with an intake of 10 g ammonium citrate orally typical episode of coma of 4 days duration occurred
7	70	♀	Diabetes mellitus Lues Cirrhosis hepatic Esophageal varices Gastro-entero-anastomosis acqv	—	Glucose I.	The patient was admitted in coma and expired within 3 days

Table I Ammonia concentration in arterial blood in patients with or without liver insufficiency

	No. of patients	Mean value µg/ml	S. D µg/ml
"Normals	26	0.90	0.24
Doubtful liver cirrhosis	24	1 19	0 46
Definite liver cirrhosis	12	1.35	0 42
Collateral circulation	15	1.54	0 46
Coma	16	3.50	1 09

Methods and material

The determination of blood ammonia was carried out by the method of Seligson (43) with some minor modifications.

The pH of the arterial blood was measured in a closed glass chamber at 37° C by a glass electrode on a pH-meter (Astrup equipment and PHM 3 Radiometer Copenhagen)

When possible the determinations were made on fasting patients in blood from the femoral artery and an antecubital vein

Ninety-three patients admitted at Bispebjerg Hospital[1] in the period 1/7 58—1/10-59 with or without liver insufficiency have been examined with regard to the ammonium concentration in the arterial and the venous blood

The patients are divided into five groups

1 Normals" no detectable disturbances in the liver function or the vascular bed attached to the liver were found.

2. Doubtful liver cirrhosis one or more points of the history or in the clinical examination suggested a possible liver cirrhosis, but no definite diagnosis of this could be made

3 Definite liver cirrhosis the diagnosis was made certain by biopsy or autopsy and at the same time no collateral circulation could be demonstrated on roentgenograms or at autopsy

[1] Dept. of Surgery A. (Head K. H. Kester M. D) Dept. of Medicin B. (Head M. Bjørneboe, M. D) and Dept. of Medicine C. (Head: N B. Krarup, M. D)

4 Liver cirrhosis with collateral circulation between the portal vein and the caval system, verified by roentgen, laparatomy or autopsy and cases with surgically established portacaval anastomosis.

5 Coma 'hepatic coma was diagnosed in cases with liver disease (history clinical signs and laboratory findings) and neurological and mental symptoms according to the following grades.

The syndrome hepatic coma has been classified according to Adams and Foley (1) in the following grades based upon the neurological symptoms Grade 1 confused and positive flapping Grade 2 slightly stuporous, drowsiness, lethargic, but easy to awake. Grade 3 deeply stuporous, only reacting to strong stimuli. Grade 4 deeply comatose, no reaction upon strong stimuli.

Results

The concentration of ammonia in arterial blood is felt to be the better index of the status of the liver because the concentration in venous blood reflects the removal of ammonia both in the liver and in the periphery

In normals" the concentration of ammonia in arterial blood has an average value of 0 90 µg NH_3-N per ml.

In liver insufficiency a small increase of the ammonium concentration in blood appears depending on the severity (table I)

Except for the group of coma where the elevation of ammonia is statistically significant ($P < 0.001$) the other groups show so much scattering in the values for ammonium concentration that no conclusion as to the condition of the liver can be drawn solely from this value (fig 1)

The group of coma contains 16 patients, 10 females and 6 males. The ages range from 53 to 76 years. In table II are given the age, sex, diagnosis, clinical course, and treatment of these patients.

Table II. Diagnosis, clinical course and treatment of patients with hepatic coma

No	Age	Sex	Diagnosis	Precipitating factor	Treatment	Course and outcome
1	63	♂	Cirrhosis hepatic Esophageal varices Chron. pyloric ulcer	Hema-themesis	Sengstaken's tube Aureomycin Glutamic acid 20 g × 2	Fall in ammonium level after glutamate. No increased arterio-venous deficit. No clinical improvement. The patient died in coma
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Table II (cont.)

No.	Age	Sex	Diagnosis	Precipitating factor	Treatment	Course and outcome
8	69	♀	Cirrhosis hepatic Esophageal varices Resectio ventriculi aeqv	Hema themesis Melaena	Glutamic acid 20 g × 2	Short increase of the arterio-venous deficit after infusion of glutamate, but no clinical improvement. Died within 8 hours after admission
9	68	♂	Cirrhosis hepatic Duodenal ulcer	—	Observation	Died after 1 day of increasing coma
10	74	♀	Chron. hepatitis Esophageal varices Gastric ulcer Thrombosis v portae	Hema themesis Melaena	Acromycin Blood transfusion Cortisone Glutamic acid 20 g ×	Some clinical improvement was seen after glutamic acid therapy but since the bleeding continued the patient expired within 4 days. Immediate fall of the ammonium concentration after glutamic acid. Positive ammonium tolerance test. The arterio-venous deficit increased
11	53	♀	Cirrhosis hepatic Esophageal varices	—	Cortisone Acromycin Glutamic acid 20 g × 2	Positive clinical effect was seen 12 hours after the infusion of glutamate together with an immediate fall of blood ammonia and a pronounced increase in the arterio-venous deficit
12	58	♀	Cirrhosis hepatic Duodenal ulcer	Hema themesis Melaena	Sengstaken tube Acromycin Glutamic acid 20 g × 3 Arginine 25 g × 2 Resectio ventriculi	Deeply comatose after operation. Infusion of glutamate resulted in slight clinical improvement, fall in blood ammonia, unaltered arterio-venous deficit for the next 12 hours. During the following arginine infusion the arterial blood ammonia increased together with the coma. Expired the next day
13	54	♀	Cirrhosis hepatic Esophageal varices Nephropathia tubul.	—	Steroids Arginine 25 g × 1 Glutamic acid 20 g × 7 Hydrochloric acid	During the development of a coma with increasing blood ammonia arginine infusion was instituted for 15 hours without any effect. Subsequent glutamate infusion causes an immediate fall of the blood ammonia, increased arterio-venous deficit and clinical improvement. While the glutamate therapy was continued the status was relatively good, but whenever the therapy was stopped the patient got worse. Died 6 days after the beginning of the coma during heavy rise of blood ammonia. Because of the alkalosis produced by the glutamate therapy hydrochloric acid was given orally (fig. 3)

Table II (cont.)

No	Age	Sex	Diagnosis	Precipitating factor	Treatment	Course and outcome
14	67	♀	Cirrhosis hepatic Esophageal varices	—	Glutamic acid 20 g × 3 Arginine 25 g × 2 Sengstaken tube	Slow developing coma transiently accentuated during a positive ammonium tolerance test. No definite effect of arginine therapy. Glutamic acid therapy was followed by significant fall in the arterial blood ammonia, decrease in the arterio-venous deficit and clinical improvement (grade 4 → grade 2). Slow elevation of the blood ammonia, resulting in coma 12 days after cessation of the glutamic acid therapy
15	[illegible]	♀	Cirrhosis hepatic Esophageal varices Thrombosis portae Pyelonefritis Hemicolectomia	Hema-themesis	Steroids	4 years history of liver disease. A single hemathemesis previous to hospitalization. Expired after 2 days of progressive coma
16	71	♀	Liver necrosis	—	Glutamic acid 20 g 3	Slowly progressive coma. Infusion of glutamic acid increased the arterio-venous deficit. The arterial blood ammonia remained high. No clinical improvement. Expired within 3 days

Fig. 1. Total ammonia in patients with or without liver insufficiency

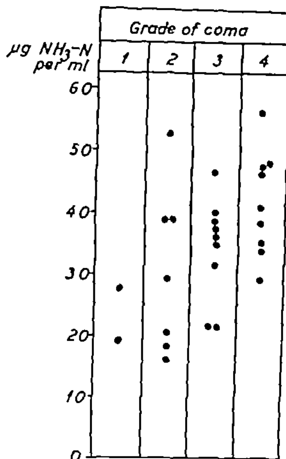


Fig 2. Total ammonia in hepatic coma

The ammonium concentrations during the episodes of coma are given in fig 1 The average value was found to be 3 50 μg NH_3 N per ml In fig 2 the arterial ammonium concentrations are plotted according to the grades of coma, and only a rough correlation was found between the degree of coma and the blood ammonium concentration.

Free NH_3 *and* NH_4 *in hepatic coma*

Up to now no differentiation has been made in this article between free NH and NH_4^+ When the term ammonia is used, it refers to both compounds, i e. the total ammonia content. In the blood the ratio between NH_3 and NH is dependent on the pH according to the following equation

$$pH = pK + \log \frac{NH}{NH_4^+}$$

where pK is 8 90 at 37 C (2, 39) This means that a rise in pH increases the free NH and vice versa.

In 31 patients pH was determined simultaneously with the ammonium determination and the free NH_3 was calculated No statistically significant difference between free NH and total ammonia could be demonstrated either in the different groups of liver insufficiency or in the different groups of coma (7 patients) although the average pH for all the groups was 0 3 pH units higher than that for the normal group

TREATMENT OF HEPATIC COMA

The treatment of hepatic coma has varied considerably between the different departments. When indicated, blood transfusion was given and tamponade of esophagus performed To abolish the absorption of nitrogen-containing substances in the intestine, most of the patients have been treated with protein withdrawal, laxative, and enema together with antibiotics (aureomycin acromycin or neomycin) Water and electrolyte balance was corrected and in a few cases corticosteroids were given in the hope of influencing the function of the liver

Treatment with glutamic acid

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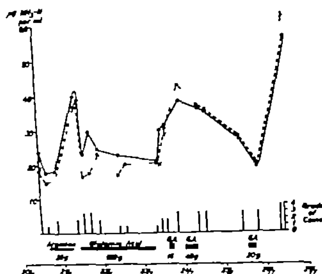


Fig. 3. The concentration of ammonia in arterial and venous blood during an episode of hepatic coma treated with arginine and glutamic acid.

— = femoral artery
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flicting. A few authors have produced good results (10, 55), but the majority are doubtful about the value (3, 13, 20, 25, 41, 46, 58) even though they do not exclude a certain effect in isolated cases.

In this investigation 10 patients were given glutamic acid intravenously (20 g sodium glutamate dissolved in 500 ml of a 5 % glucose solution). In table III the effect of the glutamic acid therapy during hepatic coma on the arterial ammonium concentration, the arterio-venous deficit, and the clinical condition are shown. From the table it appears that clinical improvement was seen in 4 patients, although only temporarily in 3 of them. Moreover it is demonstrated that this effect is apparent when a fall in the arterial ammonium concentration occurs, most often simultaneously with an increase of the arterio-venous deficit.

Characteristic of these cases was, that the higher the arterial ammonium concentration and the smaller the arterio-venous deficit, the greater was the reduction of the arterial blood ammonia by glutamic acid therapy. In other words,

Table III. Effect of glutamic acid therapy in patients with hepatic coma

Patient no.	Fall in arterial NH_4	Changes in a-v deficit	Clinical improvement
1	+	0	0
2	(+)	(+)	(+)
5	0	+	0
8	0	+	0
10	+	+	+
11	+	+	+
12	+	0	(+)
13	+	+	+
14	+	0	+
16	0	+	0

the greatest benefit of glutamic acid therapy was seen in the early acute stage of hepatic coma, where a high arterial blood ammonia and a small or no arterio-venous deficit were found.

Treatment with arginine

The treatment with arginine is based upon its participation in the ornithine cycle in the liver. The effect of arginine is as disputable as is that of glutamic acid

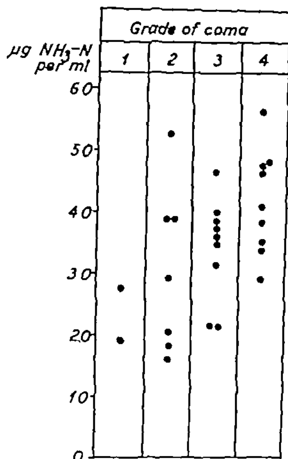


Fig Total ammonia in hepatic coma.

The ammonium concentrations during the episodes of coma are given in fig 1 The average value was found to be 3 50 μg NH_3 N per ml In fig 2 the arterial ammonium concentrations are plotted according to the grades of coma and only a rough correlation was found between the degree of coma and the blood ammonium concentration.

Free NH_3 and NH_4 in hepatic coma

Up to now no differentiation has been made in this article between free NH_3 and NH_4^+ When the term ammonia is used it refers to both compounds, i e. the total ammonia content In the blood the ratio between NH_3 and NH is dependent on the pH according to the following equation

$$pH = pK + \log \frac{NH_3}{NH_4^+}$$

where pK is 8 90 at 37 C (2, 39) This means that a rise in pH increases the free NH and vice versa

In 31 patients pH was determined simultaneously with the ammonium determination and the free NH was calculated No statistically significant difference between free NH_3 and total ammonia could be demonstrated either in the different groups of liver insufficiency or in the different groups of coma (7 patients) although the average pH for all the groups was 0.3 pH units higher than that for the normal group.

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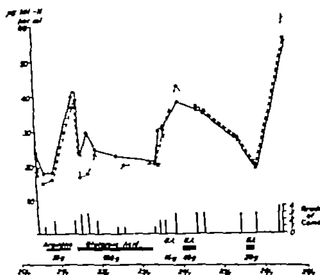


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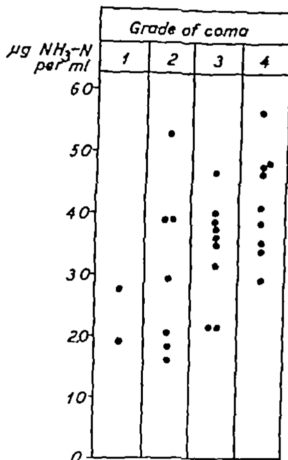


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Previously published articles concerning the value of glutamic acid therapy in hepatic coma are very con-

metabolism to a certain extent is adapted (7 15) to the increased offer of ammonia by greater availability of detoxifying agents, notably glutamic acid. As the elevated ammonium concentration indicates, this adaptation is rather limited. Infusion of glutamic acid is now unable to alter the ammonium concentration in arterial and venous blood thus other factors besides glutamic acid are limiting for the process.

If this hypothesis is correct, it raises the question whether the toxicity of ammonia is due to the removal of glutamic acid or intermediates in the citric acid cycle. The importance of a fall in the arterial ammonium concentration for the clinical effect of glutamic acid seems to indicate a more direct action of ammonia on the nervous tissue.

Arginine therapy was ineffective in the 5 patients tested. If the high blood ammonium level is due to decreased ammonia detoxication in the impaired liver an improvement is hardly to be expected by giving arginine.

Another therapeutic possibility which, however was not included in this investigation is γ-aminobutyric acid. It is reported to have some clinical effect although it does not lower the blood ammonia (13) This observation together with the knowledge of γ-aminobutyric acid as a potent metabolite in nervous tissue again points to a more direct action of ammonia on the nerve cells.

One of the main reasons why doubt is still raised about ammonia as the precipitating factor in hepatic coma, is the poor relationship between the concentration of ammonia in the blood and the grades of hepatic coma, which has been demonstrated by several previous authors (37 42, 51) as well as here.

It is known that the nerve cell membrane is more permeable to free NH_3 than to NH_4^+. Therefore free NH_3 should be more deleterious to the brain cells (28, 47 57) This has led to the anticipation of a better correlation of the degree of coma with free NH_3 than with total ammonia. Since the formation of free NH_3 is dependent on the pH of the blood, the following facts supported this theory

Wanamee and co-workers (56) found in patients with hepatic coma a respiratory alkalosis which, according to the above idea, should enhance coma. Others (29 39) were able to produce coma by giving alkalosis-producing substances. On the contrary Eiseman and Clark (16) were able to produce coma in dogs by administering ammonium salts, irrespective of the pH obtained.

In this investigation, no closer relationship between ammonia and the degree of coma was found on calculating the free NH_3.

Looking at the temporal relation between the concentration of ammonia in the blood and the development of coma, the maximal ammonium concentration in blood is reached before the coma is fully developed In 4 patients it has been possible to follow the blood ammonium level during the development of an episode of coma. It was found that the concomitant coma is most pronounced 12—14 hours after the peak of blood ammonia, as shown in fig 3 With this primary elevation of ammonium concentration, the ammonia when above a certain level may well be toxic to the brain and produce, directly or indirectly disturbances in the brain metabolism. Unlike the short reversible elevation of the ammonia as found in some cases, the metabolic disturbances which produce the encephalopathy persist even after the

Some authors (21 23 30 33) have shown a fall in blood ammonia and sometimes clinical improvement after arginine therapy in hepatic coma. Other authors cannot confirm these results (18 63)

Three patients (nos. *12, 13 14*) were given arginine intravenously (25 g L-arginine hydrochloride in 500 ml of a 5 % glucose solution) No effect of the therapy could be seen either on the ammonium concentration or in the clinical status. Fig *3* shows the different effects of glutamic acid and arginine in a patient in hepatic coma.

Discussion

It is a well known fact that the ammonium ion is strongly toxic to the nervous tissue

Theoretically the toxic effect of ammonia on brain tissue can be explained by the fact that ammonia, on entering the brain, combines with *a* ketoglutaric acid or glutamic acid and thereby removes these two important substances from the metabolism of the brain.

In experiments it *has* been shown that the citric acid cycle in which *a*-ketoglutaric acid plays an integral part, is disturbed by ammonium intoxication *and in hepatic coma, the oxygen con* sumption of the brain being decreased *(19 60)* and an accumulation of pyruvic acid and a reduction in *a* ketoglutaric acid being found (12) Since glutamic acid *is an important link in the degrada* tion of the proteins and is essential for the transportation of ions, especially of K *through the nerve cell membrane (27)* ammonia may also secondarily affect these processes.

In the acute stages of hepatic coma, the ammonium concentration in the blood attains 3 to 5 times the normal fasting level.

The removal of ammonia in extra hepatic tissue has been shown by Bessman and others (4 5 59) who demonstrated in patients with hepatic coma and elevated blood ammonia an arterio-venous deficit over the extremities and the brain.

In giving substances which release ammonia into the blood to patients with liver insufficiency or surgically established porta-caval shunt it is in some cases possible to produce episodes quite similar to those of coma (9 *38 42 62*) including electroencephalographic changes (*11* 24)

Similar observations were made in this investigation. In 16 patients with hepatic coma, a significant increase in the blood ammonia was found A pronounced arterio-venous deficit was seen in 7 patients, 5 showed a slight deficit and 4 no deficit. In 2 patients the administration of 5—10 g ammonium citrate produced typical episodes of hepatic coma simultaneously with an increase in the blood ammonia.

Glutamic acid therapy was in 8 out of 10 patients able to alter the ammonium concentration in the blood but only in the cases where the arterial ammonium concentration decreased was a clinical effect seen. *As mentioned earlier* this effect was most pronounced in the early acute stage of hepatic coma, where a *high* arterial ammonium concentration and a small arterio-venous deficit were found. *This indicates that the periphery is unable* to metabolize the sudden influx of ammonia, possible because of lack of *metabolizing* agents such as glutamic acid This substance may therefore help in these cases

On the contrary the cases with gradually rising ammonium concentration with or without coma, often show a greater arterio-venous deficit. Here the peripheral

metabolism to a certain extent is adapted (7 15) to the increased offer of ammonia by greater availability of detoxifying agents, notably glutamic acid As the elevated ammonium concentration indicates, this adaptation is rather limited. Infusion of glutamic acid is now unable to alter the ammonium concentration in arterial and venous blood thus other factors besides glutamic acid are limiting for the process.

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blood ammonia declines and thereby partly explain the poor relationship between the degree of coma and the simultaneously found ammonium concentration in the blood

Since the determination of blood ammonia in patients admitted in hepatic coma usually takes place several hours after the provoking factor has operated the value obtained may not correspond to the maximal ammonium concentration which is toxic to the brain.

Summary and conclusion

The ammonium concentration in the arterial and venous blood was determined in 93 patients with or without liver insufficiency and in hepatic coma. The average values were found to be 0 90 μg NH_3-N per ml in "normals and insignificantly elevated in liver insufficiency but with a *tendency to increase with* increasing severity of the insufficiency In hepatic coma the average value was 3 50 μg NH_3-N per ml blood

The rôle of ammonia as the precipitating factor in hepatic coma is emphasized based upon previous and present observations although the poor relationship between the degree of coma and the ammonium concentration as found by others was confirmed Calculation of the free ammonia in blood by determining the blood pH did not correct this poor correlation The temporal displacement of the maximum of blood ammonia in *relation to hepatic coma together with* more permanent disturbances of the nervous tissue, may give a reasonable explanation for this disagreement. After all the determination of blood ammonia is found of great value in the diagnosis of coma of hepatic origin.

Glutamic acid therapy was given to 10 patients. In 6 cases a decline in ammonium concentration was observed but only in 4 of these cases was clinical improvement seen. Nevertheless, glutamic acid therapy may be warrant trial in the early acute stage of hepatic coma if the blood shows a high ammonium concentration and a small arterio-venous deficit.

Arginine therapy used in 3 patients gave no improvement in the blood *ammonium concentration or in the* clinical condition.

An adaptation of the peripheral capacity for ammonium detoxication probably occurs in coma of gradual onset. In these cases glutamic acid therapy is ineffective. It is emphasized that the toxic action of ammonia on nervous tissue is presumably directly on the nervous tissue, although the mechanism is still open to question.

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From the Municipal Hospital, Arnhem, the Netherlands

Bilirubin Monoglucuronide (Pigment I): A Complex

By

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with the technical assistance of Miss A. TH. RUBEN

By application of reverse-phase column chromatography Cole, Lathe and Billing (10) made it possible to distinguish between bilirubin on the one hand and, on the other its two glucuronic acid derivatives, bilirubin monoglucuronide (pigment I) and bilirubin diglucuronide (pigment II).

These pigments have since played an important rôle in biological experiments and in clinical medicine. After intravenous infusion of bilirubin in rats, Weinbren and Billing (1) found conjugated bilirubin in the serum but only in the form of pigment I.

In their experiments on hepatectomized dogs, Bollman (2, 3), Hoffman et al. (4) and Schoenfield et al. ( ) demonstrated the formation of pigment I only. They advanced the theory that the monoglucuronide can be formed in the liver and extrahepatically whereas the diglucuronide is exclusively formed in the liver (2, 3, 4).

On the basis of this theory it was also suggested that the (increased) presence of pigment I in the serum in jaundiced patients is a result of inability of the liver cells to convert the monoglucuronide into diglucuronide. The view that bilirubin monoglucuronide exists as a chemical compound is almost universally accepted as established, and it determines to a considerable extent the prevalent views on the bilirubin metabolism and the resulting clinical conclusions (2, 3, 4, 6, 7, 8, 9), despite the fact that this chemical structure is doubted in a few instances in the literature. It was suggested that pigment I might be a complex of unconjugated bilirubin and bilirubin diglucuronide.

As early as 1954 Cole, Lathe and Billing (10) stated: "Moreover a transformation of pigment I into pigment II and bilirubin can be produced by warming and evaporation." Later Billing, Cole and Lathe (11) considered the possibility of the existence of a complex of billirubin and pigment II, but favoured the monoglucuronide-structure.

In 1958 Billing and Lathe (12), however stated that further information was needed to settle this point, thus leaving the question open. Recently Nosslin (13) also expressed doubt as to existence of

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Table II. Pigment II dissolved in normal serum. Determination of the percentage value of the three pigments at chromatography immediately after dissolving and after 4½ hours' incubation at 37° C. The Rf of all bands was determined according to Billing (19). All proved to correspond with the values reported.

Column I	Column II		Column III		Column IV	
Pigment II	Immediately after dissolving		After 4½ hours incubation at 37° C		Re-chromatography after drying of pigment I from column III	
Jaundiced serum	Bilirubin	14%	Bilirubin	24%	Bilirubin	29%
	P I	10%	P I	26%	P I	39%
	P II	76%	P II	50%	P II	32%
Bile	Bilirubin	14%	Bilirubin	40%	Bilirubin	21%
	P I	6%	P I	27%	P I	46%
	P II	80%	P II	33%	P II	33%
Jaundiced urine	Bilirubin	17%	Bilirubin	25%	Bilirubin	30%
	P I	10%	P I	20%	P I	47%
	P II	73%	P II	55%	P II	23%

raphy) for 5 min. at room temperature at pH 11.9 (adjusted with NaOH) subsequently acidified up to pH 6.0 and then submitted to chromatography. Three bands were revealed, as bilirubin, pigment I (glucuronic acid: bilirubin ratio 1:1) and pigment II. For comparison, a similar pigment II solution was kept at pH 6.0. Then only pigment II was found at chromatography. When this pigment I, formed by alkaline hydrolysis in water at pH 11.9 was extracted from the column with alcohol, dried in vacuum and again submitted to chromatography (as previously described) no difference in behaviour was found between pigment I directly obtained from bile, and this preparation obtained by hydrolysis. Both preparations showed identical behaviour in that they disintegrated into the three above mentioned bands. The chromatographic pattern obtained with P. II from urine was less well-defined than that with P. II from serum or bile. At experiments in normal human serum (pH 7.8) it was found that pigment I can also be formed in ample quantity when pigment II is added to this fluid, the mixture being left during 4.5 hours at 37° C and then submitted to chromatography by the usual method (table II, column III). Chemical analysis of such pigment I revealed a glucuronic acid: bilirubin ratio of 0.9.

As a blank experiment, a similar solution of pigment II in serum was directly submitted to chromatography. Apart from a little bilirubin (constituent of normal serum) pigment II was chiefly visible in addition to some pigment I (table II, column II).

Using a 5% aqueous albumin solution (pH 7.8) instead of serum, we found that only a small quantity of pigment I was formed. This indicates that serum constituents promote the formation of pigment I from pigment II. In this connection we have in mind enzymatic processes or more likely a catalytic influence of serum colloids (15). When the albumin is left out the aqueous solution (phosphate buffer pH 7.8) of pigment II shows no demonstrable formation of pigment I or bilirubin after 4.5 hours at 37° C.

It was demonstrated that the mixing of an increasing quantity of bilirubin with an equal quantity of pigment II in the serum, led to an increase in the quantity of complex. The analysis was carried out immediately after mixing (table III).

When the action of the alkaline milieu was longer protracted, the pigment II used in our experiments was quantitatively disintegrated into bilirubin and glucuronic acid.

Table I Glucuronic acid bilirubin ratio in the various pigments

"Mother" pigment I	Bilirubin formed +	Remaining pigment I +	Pigment II formed
Not determined		1 1	2.0
Not determined		1.2	2.2
0.8		1.2	2.2
0.9		1 0	1 7

the monoglucuronide and presented a number of arguments in favour of the complex theory Though favouring the view that pigment I is a complex of bilirubin and pigment II he concluded as well that the "elucidation of the nature of pigment I requires further investigations

From the literature it is therefore by no means certain that bilirubin monoglucuronide exists. With the following experiments we tried to bring in further arguments to solve this intriguing problem.

Methods

A. DISINTEGRATION OF PIGMENT I

We repeated the following experiments already performed by the afore mentioned investigators, and obtained thereby similar results. At quantitative determination with the Van den Bergh reaction, pigment I behaves as a mixture of bilirubin and bilirubin-diglucuronide rather than as a separate pigment (11)

Unlike pigment II pigment I appears on the column as a vaguely defined band which shows tailing — a phenomenon indicating the labile character of the substance. On extraction with alcohol or acetone, however all the pigment is dissolved. After evaporation of these solvents in vacuo at room temperature, part of the residue can be dissolved in chloroform the remainder is readily soluble in water and immediately reacts with diazotized sulphanilic acid. The chloroform-soluble part is found to consist largely of bilirubin, while the water-soluble part is chiefly bilirubin diglucuronide.

By drying the solution in a vacuum at room temperature, therefore, pigment I can be readily divided into the component parts, viz bilirubin and bilirubin diglucuronide.

New experiments on the disintegration of pigment I

Disintegration can also be demonstrated as follows by column chromatography An alcoholic solution of pigment I (obtained from human jaundiced serum by chromatography) is dried in a vacuum at room temperature, and dissolved in the polar phase of system B (10) it is then placed on a column. Three bands are found to have formed. The upper band is bilirubin the vaguely defined middle band is the remaining pigment I and the lower band is pigment II Proof of this is obtained by determination of the bilirubin and the glucuronic acid content (14) (table I)

The pigment of the middle band is then again submitted to the same procedure. At chromatography three bands again become visible.

The above mentioned experiments permit of no other conclusion than that pigment I is a complex which readily disintegrates and which consists of one molecule of bilirubin and one molecule of bilirubin diglucuronide.

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It can be regarded as known that combination of bilirubin and pigment II in an aqueous milieu yields no result. Nosslin (13) added crystallized bilirubin and pigment II from jaundiced urine to a serum free of pigment at chromatographic examination, he found a weak but unmistakable band of pigment I Repeating this experiment in careful conjunction with further particulars given by the author (personal communication) we were unable to demonstrate definite formation of pigment I

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When the action of the alkaline milieu was longer protracted, the pigment II used in our experiments was quantitatively disintegrated into bilirubin and glucuronic acid.

Table III. Relative proportion of pigment I formed in vitro in human serum, dependent on the weight ratio between bilirubin diglucuronide and bilirubin

Mixture of normal serum with P II from bile and bilirubin in the ratio	Quantity of pigment I formed determined immediately after mixing
20 : 1	1
20 : 10	1.9
20 : 50	3.5

The quantity of pigment I formed in normal serum with 10 mg/100 ml bilirubin diglucuronide, determined immediately after mixing, was considered to equal 1.

Conclusions

On the basis of the observations already made by others and on those of our own on the disintegration and the formation of pigment I, we are convinced that pigment I should be regarded as a labile equimolecular complex of bilirubin and bilirubin diglucuronide. This means that when bilirubin and bilirubin diglucuronide are together in a suitable milieu, parts of the two substances bind to form a complex of one molecule bilirubin and one molecule bilirubin diglucuronide. At chemical analysis, however (bilirubin : glucuronic acid ratio) this complex appears as bilirubin monoglucuronide. Human serum was found to be a suitable milieu for in-vitro formation of this complex.

Discussion

The concept of bilirubin monoglucuronide being a complex, has its repercussions on the explanation of bilirubin metabolism.

This is what we suggest against the current view that bilirubin monoglucuronide is formed extrahepatically (and intrahepatically: Hoffman et al. (4)) and converted into bilirubin diglucuronide exclusively by the liver. In our opinion conjugation of bilirubin — both intrahepatically and extrahepatically — always yields bilirubin diglucuronide. The fact that bilirubin diglucuronide can indeed be formed extrahepatically was established by Hoffman et al. (4) in hepatectomized rats, and by our own experiments in eviscerated rabbits and rabbits submitted both to evisceration and to nephrectomy. Quantitatively, however, the capacity of the extrahepatic tissues in this respect is poor as compared with that of the hepatic tissue (16).

The various findings obtained in animal experiments and clinical investigations can then be explained as follows.

At hepatectomy, unconjugated bilirubin accumulates in the blood. Part of this is conjugated to bilirubin diglucuronide. The simultaneous presence of unconjugated bilirubin and bilirubin diglucuronide in the blood results in the formation of the complex (which has been interpreted as bilirubin monoglucuronide). It depends on the quantitative ratio and the concentration of the two mother substances whether after formation of the complex, the remaining quantity of bilirubin or bilirubin diglucuronide is sufficient to permit of demonstration of one or both.

Infusion of bilirubin in animals (hepatectomized or not) yields similar results but more rapidly and quantitatively more marked.

Estimation of the amounts of pigment I and pigment II in the serum of jaundiced patients showed that pigment I predominated in the case of jaundice from parenchymal damage. In contrast with this, pigment II was chiefly found

in the case of extrahepatic obstruction In the case of more protracted obstruction of the bile passages, however pigment I started to show a relative increase. These conditions were confirmed by animal experiments (2, 3 4)

On the basis of the complex conception the following explanation can be offered.

When the hepatic parenchyma is injured, unconjugated bilirubin is retained in the blood this is not or hardly the case in brief transient occlusion of the extrahepatic bile ducts. The bilirubin diglucuronide which regurgitates in jaundice from parenchymal damage, will encounter much unconjugated bilirubin in the blood this affords possibility of formation of a considerable quantity of complex. In brief transient occlusion of the bile ducts, however there is no or only a slight increase in the quantity of unconjugated bilirubin and only a small quantity of complex can consequently be formed. When the occlusion is of longer duration, damage of the hepatic parenchyma occurs, and this leads to retention of unconjugated bilirubin. The presence of more unconjugated bilirubin ensures the formation of more complex (see also Nosslin (15) chapter IV)

Whenever bilirubin diglucuronide regurgitates, moreover it is possible that this is partly decomposed in the blood into bilirubin and glucuronic acid (see Methods) as result of which bilirubin becomes available to form the complex with the remaining bilirubin diglucuronide.

According to current views, the presence of pigment I in the serum signifies the inability of the liver to convert bilirubin monoglucuronide into diglucuronide. Our view is that the presence of pigment I is merely a secondary phenomenon which occurs when in the presence of bilirubin diglucuronide in the blood a sufficient quantity of unconjugated bilirubin is available to cause the formation of the complex.

In normal human bile, pigment II is chiefly found none or only a moderate quantity of pigment I and a small quantity of bilirubin (2, 10 17) or no bilirubin at all (4) Such pigment I as is present can have been formed either because some bilirubin was secreted by the liver into the bile or because part of the bilirubin diglucuronide contained in the bile was disintegrated to bilirubin. As a result, bilirubin and pigment II are again simultaneously present, and the complex can therefore be formed

The following experiment demonstrates that considerable quantities of bilirubin and pigment I can in fact occur in the bile.

Intravenous injection of bilirubin is followed by a change in the composition of the drain bile. Whereas the bile initially contained chiefly pigment II and only a moderate quantity of pigment I the bile produced after intravenous injection of bilirubin contains a relatively much larger quantity of pigment I in addition to a moderate quantity of free bilirubin. This was demonstrated by Hoffman et al. (4) in rats and dogs, and by Schalm et al. in rabbits and men (16 18) A possible explanation is that, when the liver is subject to this severe stress, it not only secretes bilirubin diglucuronide into the bile but also bilirubin and to such an extent that a large quantity of complex can be formed.

Another possibility is that the complex is formed in the liver cells, after transformation into bilirubin diglucuronide of part f the bilirubin taken up from th blood. The complex is then secreted as

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This is what we suggest against the current view that bilirubin monoglucuronide is formed extrahepatically (and intrahepatically Hoffman et al. (4)) and converted into bilirubin diglucuronide exclusively by the liver In our opinion conjugation of bilirubin — both intrahepatically and extrahepatically — always yields bilirubin diglucuronide. The fact that bilirubin diglucuronide can indeed be formed extrahepatically was established by Hoffman et al. (4) in hepatectomized rats, and by our own experiments in eviscerated rabbits and rabbits submitted both to evisceration and to nephrectomy Quantitatively however the capacity of the extrahepatic tissues in this respect is poor as compared with that of the hepatic tissue (16)

The various findings obtained in animal experiments and clinical investigations can then be explained as follows.

At hepatectomy unconjugated bilirubin accumulates in the blood Part of this is conjugated to bilirubin diglucuronide. The simultaneous presence of unconjugated bilirubin and bilirubin diglucuronide in the blood results in the formation of the complex (which has been interpreted as bilirubin monoglucuronide) It depends on the quantitative ratio and the concentration of the two mother substances whether after formation of the complex, the remaining quantity of bilirubin or bilirubin diglucuronide is sufficient to permit of demonstration of one or both

Infusion of bilirubin in animals (hepatectomized or not) yields similar results but more rapidly and quantitatively more marked

Estimation of the amounts of pigment I and pigment II in the serum of jaundiced patients showed that pigment I predominated in the case of jaundice from parenchymal damage. In contrast with this, pigment II was chiefly found

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Karolinska Hospital, Stockholm, Sweden

Lipid Metabolism and Trauma

I. Plasma and Liver Lipids During 24 hours after Trauma with Special Reference to the Effect of Guanethidine

By

Lars A. Carlson and Sten-Otto Liljedahl

Studies on metabolic changes after trauma and shock have usually neglected the lipids in plasma and especially the lipids in the tissues. The plasma lipids have been studied to some extent in different experimental animals (1 2, 3 4 5) after trauma and different results have been obtained Wadström has pointed out, however that different animals might react differently to trauma with regard to fat metabolism (4) One of the most important findings to date with regard to lipid metabolism after trauma seems to be that the plasma free fatty acids (FFA) increase significantly after trauma (4) In a controlled study in man, where the effect of semistarvation was taken into account, Wadström has demonstrated that the FFA was increased on the day following a cholecystectomy (6) This increase was apparently to some extent related to the trauma per se and to some extent to semistarvation. The other plasma lipid fractions have not been extensively studied after trauma in man. Man et al. found that cholesterol, phospholipids and the fatty acids of neutral fat decreased following operative trauma in man (7) and Wadström recorded a decrease of glycerides and phospholipids after cholecystectomy (6) It is of interest in this connection that during the course of a myocardial infarction the serum cholesterol levels have been found to decrease (8, 9) We have observed in man that the plasma lipid changes during the acute phase of a myocardial infarction (10) are almost identical with those after severe trauma, e. g burns (11) During the initial phase after the trauma there is an increase of FFA and a decrease of cholesterol, phospholipids and glycerides.

Tissue lipids have attracted little attention in connection with trauma. It is known that the glyceride stores in the adipose tissue are diminished after trauma, and Moore et al. have suggested

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such into the bile. According to this (probably emergency) mechanism which can be highly effective only half of the bilirubin to be excreted needs conjugation into diglucuronide. The likelihood of the latter possibility is demonstrated by a patient with intact liver function to whom bilirubin was administered intravenously. Fresh drain bile from this patient was examined immediately before pigment II could have been appreciably hydrolysed a considerable quantity of pigment I was found in this bile (18). Apparently this phenomenon occurs only when increased demands are made upon the secretory capacity of the liver as is the case after intravenous injection of bilirubin. After all the scanty investigations into the ratio between pigment I and pigment II in the bile in diseases of the liver revealed no deviations from the normal pattern (4, 17).

It seems theoretically possible however that severe haemolysis in adults may be associated with an increased quantity of pigment I in the bile, because the condition prevalent in these cases is analogous to that prevalent after bilirubin administration.

Summary

Experiments on the disintegration and the formation of the so-called bilirubin monoglucuronide or pigment I led to the conclusion that this pigment I is not a chemical entity bilirubin monoglucuronide but a labile equimolecular complex of bilirubin and bilirubin diglucuronide.

In vitro experiments demonstrated that human serum is a suitable milieu for the formation of such a complex. Pigment I can be obtained as well by alkaline hydrolysis in an aqueous solution of bilirubin diglucuronide. An explanation on the basis of this conception of a complex, is given for the results of the animal experiments and clinical situations in which the presence of pigment I has been demonstrated and mentioned in the literature so far.

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Tissue lipids have attracted little attention in connection with trauma. It is known that the glyceride stores in the adipose tissue are diminished after trauma, and Moore et al. have suggested

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Table I Plasma lipids before trauma and the changes of the plasma lipids found 24 hours after trauma in

	Glycerides mM/l		Cholesterol mg/100 ml
	Control	Guanethidine	Control
Before	0 48 ± 0.08	0.31 ± 0.08	134 ± 16
24 hours change from initial value	+0.17 ± 0.10	+0.06 ± 0.01	−9 ± 9
P	—	< 0 01	—

The changes were calcul ted by averaging the indi idual changes.
Statistical significance of the changes. Indicated onl when P < 0.05.

that a reduction of the adipose tissue gl cerides occurs to a greater extent after surgical trauma than during comparable starvation (12) It is kno n that the plasma FFA are derived mainl from the adipose tissue and we also know that FFA probably is the major form in which the adipose tissue glyc erides are delivered to the circulation during mobilisation of fat (13) It thus appears reasonable to connect the findings of increased FFA and decreased mass of adipose tissue after trauma The factor(s) responsible for these changes in lipid metabolism after trauma are not known in detail but the role of the cathecolamines in effecting these changes needs close consideration. The cathecolamines are known to be potent stimu lators for the release and mobilisation of FFA from adipose tissue (14 15 16) It is also ell known that trauma initiates an increased release of cathe colamines (17 18)

From these different aspects it was considered worthwhile to start a more detailed stud on trauma and lipid metabolism Since the liver is known to extract FFA in amounts proportional to the concentration in plasma (19 20 21) and subsequently to esterify the extracted FFA to glycerides and phospholipids (22 23 24) the liver and plasma lipids were investigated in this first study To assess the role of the sympathetic nervous system in producing the changes observed the effect of guanethidine, a periferall acting sympathetic inhibitor (25) on the traumatic changes was also studied The results of these studies prompted investigation of the cellular localisation of lipids after the trauma and after administration of norepinephrine.

Material and methods

Animals and experimental procedure

Adult mongrel dogs weighing around 15 kg were used after a fasting period of 20—24 hours. The dogs were anesthetized with Nembutal ® 30 mg/kg bod weight. Liver biopsies were obtained through a midline incision. The pieces of liver taken generally weighed 200—400 mg The liver was carefully sutured and the abdominal wall closed. In the first experiments additional trauma was inflicted b fracturing the tibia. It was soon realised ho ever that a li er biopsy in itself was a sufficient trauma and that the magnitude of response varied from animal to animal in a ay which appeared unrelated to the coexistence of fracture. Latterly therefore trauma was inducted merel b the li er biopsies. The animals were kept anesthetized 8 hours after the first trauma, receiving a slow infusion of saline After 8 hours the infusions were stopped and the animals allowed to wake up. They were again anesthetized 12 hours later The final samples were taken 24 hours after beginni g the

6 control dogs and 4 guanethidine-treated dogs. Mean value ± standard error of the mean

Cholesterol mg/100 ml	Phospholipids mg/100 ml		FFA mEq/l	
Guanethidine	Control	Guanethidine	Control	Guanethidine
106 ± 18 −14 ± 6 —	276 ± 27 +17 ± 11 —	231 ± 33 −13 ± 20 —	0.60 ± 0.09 +0.40 ± 0.06 < 0.01	1.75 ± 0.23 −1.12 ± 0.26 < 0.01

Table II. Liver lipids before and the changes of the liver lipids found 24 hours after trauma in 6 control dogs and 4 guanethidine-treated dogs. Mean value ± standard error of the mean

	Glycerides µM/g liver		Cholesterol mg/g liver		Phospholipids mg/g liver	
	Control	Guanethidine	Control	Guanethidine	Control	Guanethidine
Before	6.5 ± 1.6	7.9 ± 1.4	2.78 ± 0.53	2.90	24.1 ± 2.8	29.7 ± 2.5
24 hours change from initial value	+8.4 ± 2.2	+0.1 ± 0.9	+0.60 ± 0.50	+ 0.10	+2.4 ± 2.4	−2.2 ± 7.1
P*	<0.01	—	—	—	—	—

and see table I. Values from only 2 dogs.

experiment and the animals killed. Blood samples were taken from peripheral vein.

Guanethidine[1] was administered to two dogs in a dose of 15 mg/kg body weight about 1 hour before the experiment, and in two other dogs a total amount of 15 mg/kg was given in divided doses over several days before the experiment. As far as could be judged from clinical appearance, FFA and liver glycerides in these 4 dogs there was no major difference between these two different kinds of treatment. These 4 dogs have accordingly been treated together when dealing with the results.

l-norepinephrine in saline was given to 3 anesthetized dogs at the rate of 1 µg/kg body weight per minute for a period of 12 hours. Except the anesthesia these dogs were not exposed to any trauma.

[1] Kindly supplied as Ismelin™ by A. B. Ciba, Stockholm.

Lipid analyses

The blood samples were collected in heparinized tubes, and either centrifuged immediately and the plasma extracted, or stored for some hours at +4° C. The plasma FFA was determined according to Dole (26) and the other plasma lipids as described previously from this laboratory (27). The liver samples were homogenized immediately with 5 ml of methanol in an all-glass homogenizer. Exactly 10 ml of chloroform was added and after mixing 15 ml of saline was added and the resulting two-phase system allowed to equilibrate over night. Lipid analyses were then made on aliquots of the chloroform phase as described previously (27).

Histologic techniques

Portions of the liver, lungs, heart, skeletal muscles and kidneys were taken immediately before death and were fixed in 10 % for

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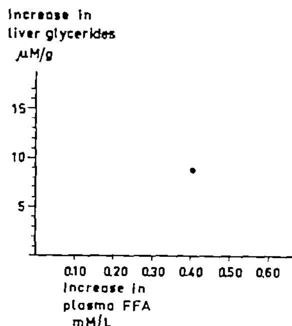


Fig 1 The relationship between increase in plasma FFA and in liver glycerides 24 hours after trauma in 6 dogs.

malin. Pieces of the liver were also taken at the various biopsy times. Specimens were frozen, sectioned and stained with Sudan IV and counterstained with hematoxylin. The sections have been studied by Dr Claes Wirsén, M D Department of Histology Karolinska Institute The detailed results of these studies will be published elsewhere (Wirsén et al.)

Results

Plasma lipids

The results from six control dogs subjected only to trauma and from 4 dogs pretreated with guanethidine and then subjected to trauma are given in table I The only significant change in the dogs subjected only to trauma was an increase in the FFA fraction. The FFA increased here from 0 60 to 1 00 mEq/l.

The dogs which had been pretreated with guanethidine had significantly higher initial FFA values than the control dogs ($P < 0\,01$) 24 hours after the trauma the FFA fraction in these dogs had decreased from 1 75 to 0 53 mEq/l ($P < 0\,01$) In the dogs given an infusion of norepinephrine the plasma FFA level increased from around 0 5 to 3 0 mEq/l within 1 hour after beginning the infusion and remained at that level during the entire infusion.

Liver lipids

In table II are recorded the results from the liver lipid analysis. It is seen that in the 6 control dogs the only significant change 24 hours after the trauma is the increase of liver glycerides. This fraction increased on an average from 6.5 to 16 μM/g The increase in liver glycerides is plotted against the increase in plasma FFA in fig 1 There seems to be a direct relationship the liver glycerides increasing linearly with the increase in FFA.

The dogs pretreated with guanethidine did not differ significantly from the untreated dogs with regard to the initial liver glyceride content. Still more interesting is that 24 hours after the trauma these dogs did not show any increase in the liver glycerides. The cholesterol and phospholipid contents of the livers in these animals did not differ from those in the untreated dogs and no changes were observed in these lipid fractions after the trauma

A more detailed analysis of the plasma FFA and the liver glycerides in 3 control and the 4 guanethidine treated dogs is given in figs. 2 and 3 In the control dogs the most rapid increase of the FFA fraction was between the second and the eighth hour The guanethidine-treated group however had a pronounced decrease of the FFA from zero to eight hours. The liver lipids increased sig-

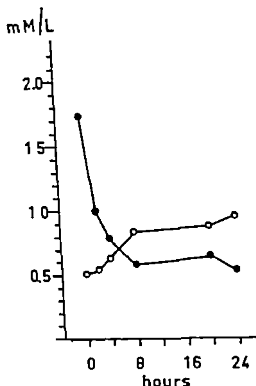


Fig. 2. Mean value of FFA concentration in 3 dogs subjected to trauma ( ) and in 4 dogs subjected to trauma after pretreatment with guanethidine (●)

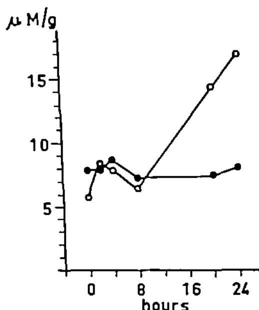


Fig. 3. Mean value of liver glyceride concentration in 3 dogs subjected to trauma (○) and in 4 dogs subjected to trauma after pretreatment with guanethidine (●).

nificantly in the control group from the eighth to the twenty-fourth hour In the pretreated group the liver glycerides did not change during the time studied.

The livers in the dogs given norepinephrine had a glyceride concentration of around 90 μM/g Although the glyceride level was not analysed before norepinephrine was given, data from other dogs indicate that this represents an increase to at least 10 times the initial level.

Histological findings

The livers of the dogs subjected to trauma had developed certain degree of fatty liver (fig 4 A) which was most pronounced 24 hours after the trauma. The dogs pretreated with guanethidine did not show any significant fatty degeneration of the liver as compared to the controls (fig 4 C) The dogs given norepinephrine had developed an extreme degree of fatty liver (fig. 4 E) The histological findings were in good agreement with the estimated amount of glycerides in the livers.

The myocardium of the norepinephrine infused dogs showed a marked fatty degeneration (fig 4 F) Some areas of fatty degeneration of the myocardium were also present 24 hours after the trauma (fig 4 B) but could not be found in the guanethidine-pretreated dogs (fig 4 D)

In the lungs the norepinephrine infused dogs showed quantitatively the same changes as the dogs subjected to

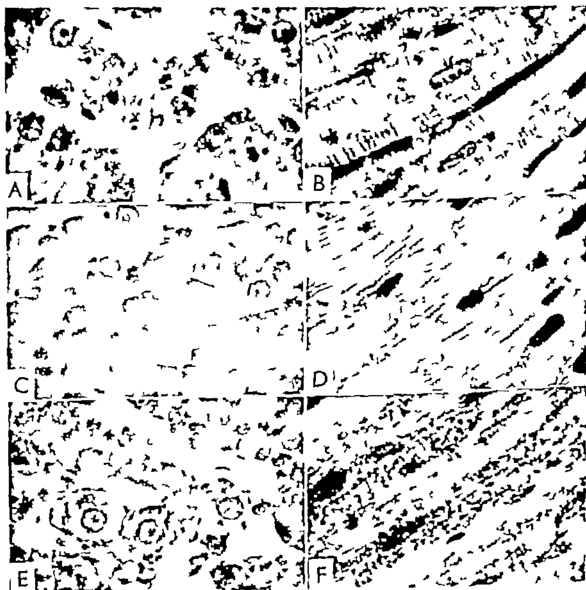


Fig. 4. A. Liver 24 hours after trauma. Small fat granules are seen in the periphery of the laminae. Sudan IV — Ehrlich hematoxylin stain. 640 × B. Heart muscle of the same animal. Only very sparse Sudan-positive granules. The same preparative method. 640 × C. Liver 24 hours after trauma in a dog pretreated with guanethidine. Sudan positive granules are very sparse and confined mainly to single cells. The same preparative method. 640 × D. Heart muscle of the same animal. The myofibrils are free from fat. The same preparative method. 640 × E. Liver 12 hours after continuous administration of l-norepinephrine to a rat of 1 µg/kg body weight per minute. Marked fatty degeneration of the liver cells. The same preparative method. 640 × F. Heart muscle from the same animal. Abundant fat granules in longitudinal rows between the myofibrils as in fatty degeneration. The same preparative method. 640 ×

trauma but the changes were much more pronounced after norepinephrine. The main findings were increased amounts of fat localised to alveolar macrophages. In the pulmonary parenchyme there was an abundance of atelectasis and cell infiltration. No pulmonary edema or extravasation of blood

was observed These findings were not observed in animals which had been given guanethidine Interestingly relatively few fat droplets were observed in the ... cells in all groups. The other organs studied showed especially after norepinephrine, similar findings with fatty degeneration of the cells.

Discussion

As expected the FFA fraction increased after trauma in conformity with previous investigations (4 6) Simultaneously a fatty liver developed This increase in liver glycerides might well be ascribed to the increased FFA level as it has been demonstrated that the liver takes up FFA in amounts related to the plasma level (19 20 21) Fig. 1 also demonstrated that the higher the increase of the FFA level was, the more increased the liver glycerides. The theoretical contribution of the increase in FFA to the liver glycerides could be roughly calculated as follows. Assume a plasma volume of 0.5 l, a fractional turnover of the FFA of 0.30/min. and a liver plasma flow of 30 per cent of the total blood volume per minute. If we have a fairly similar fractional uptake of FFA in different tissues the increase in FFA of 0 40 mEq/l (table I) would give rise to an increased liver uptake of FFA of $0\,40 \times 0.5 \times 0.30 \times 0.30 \times 60 = 1\,1$ mEq/hour That this figure probably is not an overestimate is evident from the data of Fine and Williams (21) who found a liver uptake of FFA of *1.6* mEq/hour at a portal vein FFA concentration of 0.35 mEq/l. It can also be calculated from the data of McElroy et al. that the liver uptake of FFA at portal concentration of 0.4 mEq/l was around 1.8 mEq/hour (20) From 8 to 24 hours (fig 5) the uptake of 1 1 mEq/hour would correspond to 6 mM of fatty acids calculated as triglycerides. The mean liver weight was 400 g and the mean increase in liver glycerides around 10 μM/g giving a total increase of 4 mM of glycerides. The increase in FFA might thus be sufficient to cause the fatty liver Of course the synthesis in the liver and secretion into plasma of lipoproteins rich in glycerides must be considered in this connection. It is of interest to note here that no significant increase of the plasma glycerides was observed. Analysis of the composition of the fatty acids of the isolated liver glycerides as compared with the composition of the fatty acids in the isolated FFA fraction indicated that the newly formed liver glycerides might be derived from the FFA fraction (unpublished data in collaboration with S. Landstedt)

After treatment with guanethidine which acts as a sympathetic blocking agent (25) no increase in the liver glycerides was observed and the behaviour of FFA was reversed as compared to the controls, with a pronounced decrease. The high initial FFA level in these dogs deserves a brief comment. It is known that after administration of guanethidine there is an initial increase in blood pressure (23) During this time and for somewhat longer the FFA level increases considerably (28) These phenomena might be related to a release of stored cathecolamines. Furthermore the flux of FFA through plasma might not be as high as indicated by the concentration only as the heart rate and therefore probably the blood flow was much lower in the guanethidine-treated animals than in the controls. It has been suggested that the blood flow should be considered when the total

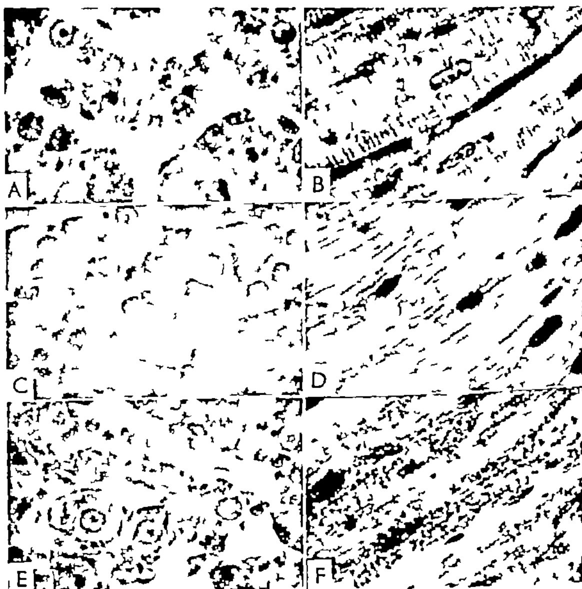


Fig 4 A. Liver 24 hours after trauma. Small f t granules are seen in the periphery of the laminae Sudan IV — Ehrlich hematoxyli stain. 640 × B. Heart muscl of the same animal. Only ery sparse Sudan positive granules. The same preparative method. 640 × C. Liver 24 hours after trauma in a dog pretreated with guanethidine Sudan-positive granules are very sparse and confined mainly to single cells. The same preparative method. 640 × D Heart muscle of the same animal. The myofibrils are free from fat. The same preparati e method. 640 × E. Liver 12 hours after continuous administration f l-norepinephrine t a rate of 1 μg/kg body weight per minute. Marked fatty degeneration of th liver cells. The same preparative method. 640 × F Heart muscle from the same animal. Abundant fat granules in longitudinal rows between the myofibrils as in fatty degeneration. The same preparative method. 640 ×

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and other organs. These findings were still more exaggerated after infusion of norepinephrine. In the pulmonary parenchyme atelectasis and cell infiltrations were found

2. The amount of fat droplets after trauma in dogs pretreated with guanethidine, observed in the liver myocardium and other organs, was smaller than in dogs subjected to trauma only

The interrelationship between increased FFA levels and fatty infiltration of various organs is discussed as well as the role played by the sympathetic nervous system in the derangement of lipid metabolism after trauma.

Acknowledgment

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flux of FFA through plasma is discussed (29) This question warrants further study however

The finding that after guanethidine treatment the plasma FFA decreased after trauma and the development of fatty liver was prevented suggests that the cathecolamines and the sympathetic nervous system have an important role in causing the deranged lipid metabolism after trauma. The question immediately arises whether a cathecolamine induced increase of lipolysis in the adipose tissue with a concomitant increased flux of FFA through the plasma room to other tissues, is a purposeful mechanism for the energy metabolism after trauma, or whether it is to be regarded as an inevitable and possibly harmful effect. The appearance of fatty liver and fatty infiltration in the myocardium and other organs observed after trauma might eventually be regarded as a harmful effect, as might the changes — similar in type but more pronounced — seen after the administration of norepinephrine If so this metabolic feature might well be related to the basic mechanisms of irreversible shock where there is an increased secretion of norepinephrine as far as can be judged from urinary analysis of cathecolamines (17 18) The development of a fatty liver in dogs after norepinephrine infusion was quite recently also demonstrated by Feigelson et al. (30) These authors were inclined to regard the increase of FFA after norepinephrine as the direct cause of the fatty liver However it has not been possible to rule out the possibility that the pressor or other effects of norepinephrine might be responsible for the development of the fatty liver in a direct or indirect way The fatty infiltration of the myocardium as well as other organs and the pulmonary changes observed here after norepinephrine infusion in dogs will be subjected to further study

The results observed here suggest that the metabolic effects of trauma and cathecolamines, especially on lipid metabolism should be carefully studied in animals and man in order to evaluate their importance for the pathogenesis of irreversible shock. The demonstration that guanethidine inhibited the increase in FFA and liver glycerides focuses our interest on the possibility that a therapeutic approach to shock might be made in the form of chemical sympathectomy

Summary

Biochemical and histological studies have been done in dogs during 24 hours after trauma with and without sympathetic blockade with guanethidine and also after infusion of norepinephrine.

The following *biochemical* observations were made

1 a) The plasma FFA increased significantly after trauma

b) The liver glycerides also increased significantly 24 hours after trauma

c) A positive correlation was present between the increase in FFA and increase of liver glycerides.

2 After guanethidine pretreatment neither the FFA nor the liver glycerides increased following the trauma.

3 The infusion of norepinephrine resulted in a sustained elevation of plasma FFA and after 12 hours the liver glycerides had increased more than 10 times.

The following *histological* observations were made

1 After trauma a fatty degeneration was observed in the liver myocardium

and other organs. These findings were still more exaggerated after infusion of norepinephrine. In the pulmonary parenchyma telectasis and cell infiltrations were found.

2. The amount of fat droplets after trauma in dogs pretreated with guanethidine, observed in the liver myocardium and other organs, was smaller than in dogs subjected to trauma only.

The interrelationship between increased FFA levels and fatty infiltration of various organs is discussed as well as the role played by the sympathetic nervous system in the derangement of lipid metabolism after trauma.

Acknowledgment

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Electroencephalographic Findings in Chronic Phenacetin Abusers

By

Antero Kalanen and Pentti Vallkala

The abuse of phenacetin is very common. About every fifth hospital patient in Denmark and Finland has taken phenacetin daily (2 6) In Switzerland about 20 000 persons are considered to be actual abusers (8) The number of persons suffering from phenacetin toxicomania is increasing in psychiatric departments the number of these cases has grown five-fold between 1944 and 1954 (3)

It is common knowledge that numerous symptoms of the central nervous system are associated with the abuse of phenacetin. The principal psychic symptoms in the initial phase are instability disordered volition and sometimes also acute psychic disturbances. Psychical symptoms are encountered in 28 per cent of all abusers (9) It has also been noted on the other hand that these patients may have suffered from personality disturbances before they became phenacetin addicts. Introversion and inhibitions occur in women and an increased need for confession in men.

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These symptoms of the central nervous system and an interesting clinical case of our own led us to study the electroencephalographic findings in long-term phenacetin abusers.

Material and method

The patient material consisted of 16 chronic phenacetin abusers under treatment at the medical department. Only patients under 40 were included in the series to obviate possible electroencephalographic changes associated with middle age and old age that might interfere with the interpretation of the results. The age range was 19—39 years, mean 30 There were 12 women and 4 men. The patients renal function and blood picture were also examined

The criterion for selection was the absence of any indication other than headache for the electroencephalographic examination. All the cases with episodes of unconsciousness or convulsions were omitted. Neurotropically acting medicines including analgesics — were withheld from the patients for 2 days before the examination.

The electroencephalographies were performed with an 8-channel and in some cases

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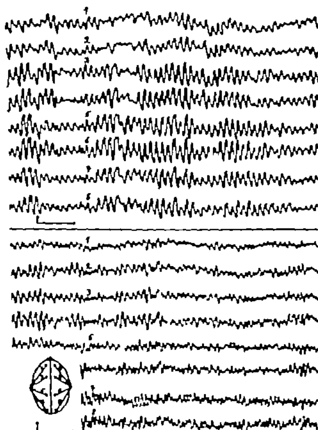


Fig. 2. Female. Age 26. *Upper record* EEG recording in the phenacetin abuse phase. Generalized 6—7 c/s activity with intermittent high voltage rhythmical runs. No alpha activity. *Lower record* EEG recording from the same patient after radical reduction of phenacetin consumption (11 months after the previous recording). Alpha activity was distinctly increased. Theta activity was diminished, appearing at times in the frontal and central regions. Voltage bar 50 U V. Time bar 1 sec.

fore the use of analgesics. The pain had been unspecific in the rest of the patients. In several patients, however psychic tension and hurry aggravated the pain. A pathological EEG pattern is not associated with the history of migraine.

The renal function of these patients was generally quite normal. Ability to concentrate was lowered in 13 and phenolsulphonphthalein excretion was low in 4 patients. Two patients showed slightly elevated serum creatinine, 2.2 and 4.1 mg respectively. None of the patients had clinical symptoms of uremia. The electroencephalographical finding was normal in the patient with the highest creatinine value. Four patients had mild anemia, which showed no correlation with the EEG findings.

The subjective symptoms were usually headache, fatigue and apathy. The patient had a definite feeling of relief after the ingestion of phenacetin, perked up and felt more ready to work. Some patients also suffered from severe anorexia. The patients showed a fairly considerable weight loss.

Case 1 The patient was nurse of 26 who had taken as much as 4.0 g of phenacetin daily for 10 years, in all 4.0 kg. She had lost approximately 10 kg in weight, her appetite had been extremely poor. She was drowsy and tired in the morning and suffered from severe headaches. Double vision occurred at times. The blood picture was normal and

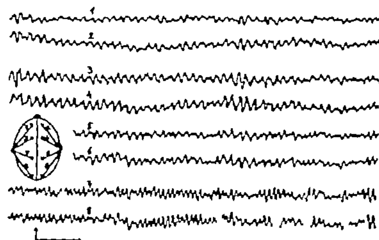


Fig 1 Male. Age 39. An EEG recording representing the most typical EEG change in this series. It shows intermittent rhythmical 5—8 c/s activity in the frontal and parietal regions parasagittally Normal alpha activity in the occipital regions. Voltage bar 50 μV Time bar 1 sec.

a 16-channel Kaiser electroencephalograph. Photic stimulation was employed as part of the examination of each patient (A Kaiser photostimulator frequency range 4—24/sec)

Results

Five of the 16 patients examined gave a normal EEG finding Eleven had an EEG which was interpreted as pathological These findings can be divided into mild changes in 5 cases and severe changes in 6 patients.

Mild changes

Group I (5 cases) Intermittent rhythmical 5—8 c/s activity in the frontal and parietal regions parasagittally Normal alpha activity in the occipital regions. This group is the most homogeneous as regards the changes (fig 1)

Severe changes

Group II (4 cases) Generalised 5—8 c/s activity with possible high voltage runs. No alpha activity

Group III (2 cases) Bilateral slow wave discharges The background activity was normal or slightly slowed.

Flicker light reaction Abnormal flicker reactions occurred in 7 cases of the total series. The reaction was evaluated as mild in 4 cases The discharges in these cases were of short duration, and generally appeared at a flicker rate of 20—24/s. Three persons gave an epileptiform flicker reaction accompanied by large-amplitude intermixed fast and slow potentials at all recording points. The patient in these cases complained that the flicker light was very unpleasant The flicker reaction was pathological independent of the degree of severity of the other electroencephalographic findings.

Clinical picture

The patients had consumed 0 8—6.0 kg of phenacetin according to an anamnestic evaluation. The average consumption was 2 0 kg of phenacetin. In general, the greater the patient's consumption of phenacetin the greater were the electroencephalographic changes established None of the patients had taken pure phenacetin all took compound powders containing salicylic acid and caffeine as well We have found no reports in the literature that these substances cause changes in the electroencephalogram.

The history of 4 of the 16 patients indicated headache of migraine type be

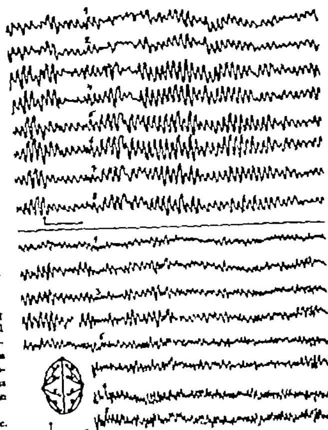


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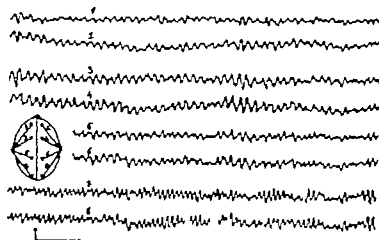


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tremely unpleasant on the first occasion, but t no longer irritated her in the second examination. Before her abuse of phenacetin was discovered the diagnosis was a hypophyseal tumour for this patient also, on the basis of the clinical picture

Discussion

We have been able to establish pathological findings in phenacetin abusers. The typical features are partial bilateral slowing and deviation of the flicker reaction. Migraine patients have also shown EEG changes somewhat similar in type. The changes demonstrable in migraine patients may be difficult to distinguish from the EEG findings of epileptics. Definite pathological EEG findings were established in 14.5 per cent of 400 patients with migraine or epilepsy (1) However manifest migraine was demonstrable in the history of only 4 of the patients of our series.

Definite EEG changes were established also in uremic conditions. Most of the cases involved unspecific slowing down of the EEG combined with reduction in lertness (4 5 7 10) The flicker light provoked pathological EEG changes in these conditions. The changes showed no correlation, however with the intensity of the uremia, and no history f phenacetin consumption was noted for these cases (4) In contrast the patients of our own series had no uremia and 2 had only mild azotemia. A pathological EEG finding was established in the majority of the cases although the renal function was quite normal.

The EEG changes were normalised or clearly diminished after the discontinuance of phenacetin. Hence it seems to us that the changes involved cannot be associated with migraine or renal disease but are specific findings associated with phenacetin abuse Further support for this is the fact that most patients have withdrawal symptoms when taken off phenacetin and all cerebral symptoms disappear later Because the abuse of phenacetin is very common, the finding made in this study is important in routine EEG work.

Summary

An electroencephalographic record was taken without specific indications from sixteen patients of the medical department who had consumed an average of about 2 kg of phenacetin. The patients mean age was 30 years and they were all under 40 Two had mild azotemia and the others only impaired ability to concentrate. Four had a history of migraine.

A pathological electroencephalogram was obtained from 11 patients. The most typical EEG change was intermittent rhythmical slow activity in the frontal and parietal regions. In severe cases the changes were generalised nd normal alpha activity was completely absent. An abnormal flicker reaction was established in 7 cases. This reaction was epileptiform in 3 patients.

A follow-up EEG was obtained after withdrawal from phenacetin from 2 patients whose clinical diagnosis had been hypophyseal insufficiency The changes had become normal or diminished clearly in 9 and 11 months.

The uthors regard the changes as associated with long-term phenacetin abuse and correlate them with the cerebral symptoms demonstrated in the patients.

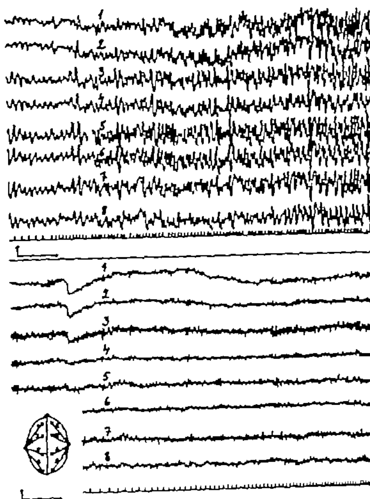


Fig 3 Female. Age 25 *Upper record* Epilept form flicker reaction in the patient who consumed large quantities f phenacetin. Th patient found the flicker light very disagreeable *Lower record.* Flicker reaction in the same patient fter the discontinuance of phen cetin (9 months after the previous record) The flicker light now caused the patient no disagreeable sensations. Voltage bar 50 uV Time bar 1 sec. Flicker pattern at the lower margin of the pictures.

renal function good The skull roentgenogram and examination of the ocular fundi were normal. The hypothetical d agnosis was hypophyseal tumour and Simmond s syndrome Attention was then paid to the patient s history of phenacetin consumption She was induced to give up analgesics and her general condition improved considerably She put on 5 kg and was again capable of work. At this time 11 months had elapsed from the earlier examination (fig 2) The anorexia and the suspicion of brain tumour obviously arose from the symptoms provoked by th use of phenacetin.

EEG changes after the discontinuance of phenacetin

A follow-up examination after the patient had given up the use of phenacetin almost completely was possible in 2 cases.

Fig 2 shows a case in which a severe EEG change was demonstrable on the first examination The second record in the same illustration presents the situation 11 months later The general theta acti ity characterised by intermittent rhythmical runs was clearly diminished. Alpha activity was increased and had become the preponderant type of activity in the occipital regions. The patient s clinical condition had also improved essentially during this time. Her weight had increased and the chronic fatigue had disappeared

Fig 3 shows an epileptiform flicker reaction which was completely absent at the follow-up examination 9 months later The patient found the flicker light ex

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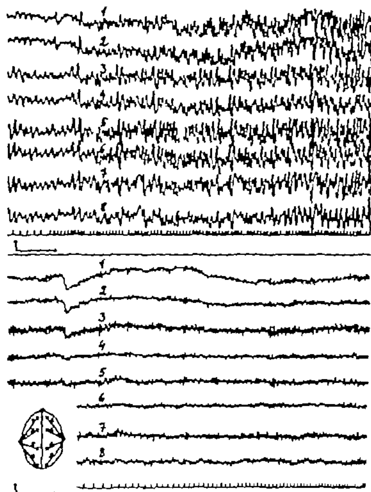


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Fig 3 shows an epileptiform flicker reaction which was completely absent at the follow up examination 9 months later The patient found the flicker light ex

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From the First Department of Medicine (Head P. I. Halonen, M. D.)
University of Helsinki, Finland

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By

R. Pelkonen and E. Pitkänen

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A hypotonic crisis in connection with pheochromocytoma has rarely been found (2, 3, 4, 5, 6, 11) except during anesthesia or after operation. In one case (6) where a hypotonic crisis was the dominant symptom the electrocardiogram suggested the presence of diffuse myocardial damage. Meagre data are available concerning the catechol amine content of the tumors in such cases. In the case of Richmond et al. (11) the main catechol amine was adrenaline.

The present case was characterized by a hypotonic crisis associated with marked electrocardiographic changes.

Case report

The patient was a 40-year-old female of middle height and an asthenic constitution, who 13 years previously had undergone thyroidectomy because of thyrotoxic symptoms. She had a normal delivery eight years before.

During the last ten years she had suffered from migrainelike attacks of headache with dizziness and nausea, lasting from ten to thirty minutes. After the attacks she had observed palpitation of the heart, sweating and hunger. A high blood pressure was recorded once. The blood pressure was normal in several control measurements. On the day of admission she developed a feeling of substernal pressure and nausea, with severe vomiting and one hour later she lost consciousness. She was immediately admitted to the hospital.

On admission the patient was unconscious, the skin was pale, the radial pulse faint, and the pulse rate 110/min. The blood pressure was 75/60 mm Hg. A noradrenaline infusion was instituted and large doses of hydrocortisone were given. The blood pressure rose to 100/60 mm Hg and the patient gradually regained consciousness. The pulse was still faint and rapid and the patient sweated excessively. Gradually she became dyspneic, râles indicating pulmonary congestion were heard, and digitalis therapy was instituted. Nevertheless the symptoms became aggravated and the blood pressure level fell gradually. The patient succumbed to pulmonary edema 30 hours after admission.

Examination of the blood revealed a white cell count of 39,000/mm³, hemoglobin 17 g/100 ml, and NPN 64 mg/100 ml. The urine gave a positive test for protein. The Nylander test for urinary sugar was negative. The serum sodium level was 156 mE/l, potassium 4.9

Submitted for publication June 18, 1962.

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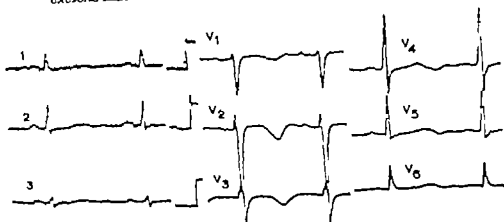


Fig. 2 Electrocardiogram in case of pheochromocytoma. A 42-year-old female. Paroxysmal hypertension with attacks of headache. A left-sided benign adrenal medullary tumor weighed 15 g and contained 5.5 mg of noradrenaline and 0.5 mg of adrenaline per gram of tumor wet weight

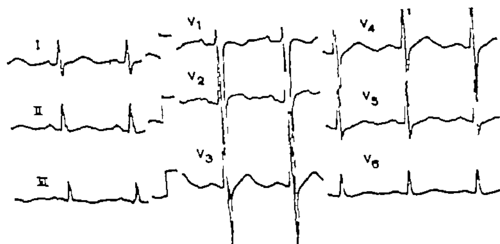


Fig 3 A 17-year-old man. Persistent hypertension (180 120 mm Hg) for least six months. Severe hypertensive changes in ocular fundi A left-sided benign adrenal medullary tumor The tumor weighed 40 g 1 contained 7 2 mg of noradrenaline with trace of adrenaline per gram of tissue, wet weight.

Chemical examination of the tumor Small samples of the tumor were homogenized in 6 perchloric acid. The catechol amine content was determined by he iodine oxidation method Paper chromatography (n-butanol acetic acid water 5 1 4) was used for further identification of the compounds The samples contained 3 8—1 1 mg of noradrenaline per gram of tissue wet weight. No adrenaline or hydroxytyramine was found.

Discussion

Electrocardiographic tracings in two other cases of pheochromocytoma are included (figs. 2 and 3) to demonstrate the changes usually encountered. In fig 2 the QT interval is lengthened and the T waves in leads V—V and aVL are negative. In the other recording (fig 3)

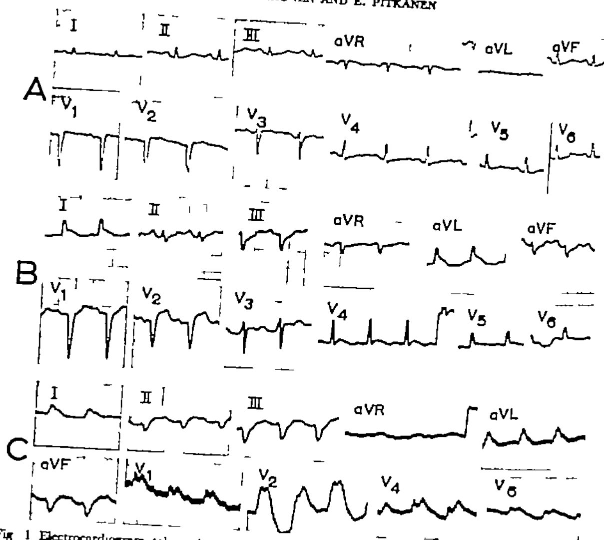


Fig 1 Electrocardiogram taken A. one hour after onset of a hypotonic crisis in a case f pheochromocytoma, B. 13 hours after onset of th crisis and C. 26 hours after onset f the crisis.

mE/l and calcium 8 2 mg/100 ml. The activity of serum G—O-transaminase was very high (234 units) and that of serum glucose-6-phosphate dehydrogenase (7) was also elevated (9 7 units)

The electrocardiograms taken 1 13 and 26 hours after onset of the attack are shown in fig 1 A—C.

First tracing (1 A) There was sinus tachycardia (124 beats/min) a slightly lengthened QT interval (0 35 sec) low voltage and a QS complex in leads V_1 and V with slight elevation of ST segments.

Second tracing (1 B) The QT interval was normal but there was a definite elevation of the ST segment in leads I, aVL, V V and a reciprocal depression of the ST segments in leads II III and aVF

Third tracing (1 C) The QRS complexes were markedly deformed.

Autopsy showed that both lungs were extremely edematous. There was a clear yellowish fluid in the pleural cavities. The size of the heart was normal and no fluid was found in the pericardium. The endocardium and the cusps were intact. The coronary arteries were patent and showed no atherosclerotic changes. The cut surface of the myocardium was pale and flabby No hemorrhagic areas were seen.

A brownish tumor was found in the area of the right suprarenal gland. It had a diameter of 6 cm and contained numerous hemorrhagic reas. Histologically it was a benign pheochromocytoma.

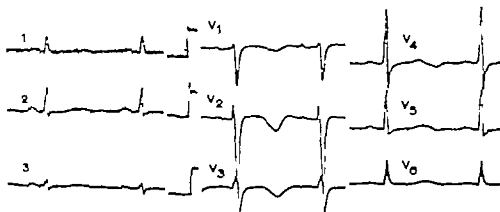


Fig. 2. Electrocardiogram in case of pheochromocytoma. A 42-year-old female. Paroxysmal hypertension with attacks of headache. A left-sided benign adrenal medullary tumor weighed 15 g, and contained 3.5 mg of noradrenaline and 0.5 mg of adrenaline per gram of tissue, wet weight.

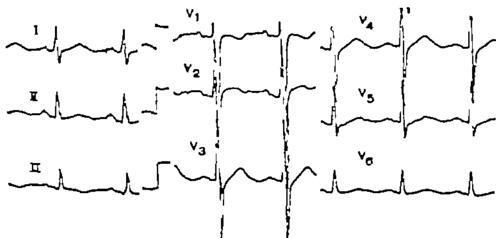


Fig. 3. A 17-year-old man. Persistent hypertension (180/120 mm Hg) for at least six months. Severe hypertensive changes in ocular fundi. A left-sided benign adrenal medullary tumor. The tumor weighed 40 g. It contained 7 mg of noradrenaline with trace of adrenaline per gram of tissue, wet weight.

Chemical examination of the tumor. Small samples of the tumor were homogenized in 6 perchloric acid. The catechol amine content was determined by the iodine oxidation method. Paper chromatography (n-butanol acetic acid water 5 1 4) was used for further identification of the compounds. The samples contained 3.8—1.1 mg of noradrenaline per gram of tissue wet weight. No adrenaline or hydroxytyramine was found.

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Electrocardiographic tracings in two other cases of pheochromocytoma are included (figs. 2 and 3) to demonstrate the changes usually encountered. In fig. 2 the QT interval is lengthened and the T waves in leads V—V and aVL are negative. In the other recording (fig. 3)

a slightly lengthened QT interval is associated with signs of left ventricular hypertrophy.

Remarkable in the present case was the hypotonic crisis resistant to all therapy and the massive electrocardiographic changes. The changes suggested the presence of myocardial infarction. At autopsy, however, diffuse myocardial damage was found.

The hypotonic crisis was evidently the result of myocardial damage due to acute intoxication by noradrenaline from the tumor. Adrenaline, on the other hand, was the main component in a similar case of Richmond et al. (11). Both substances are known to produce electrocardiographic changes in experimental animals when administered in large doses (8).

Hemorrhage in a pheochromocytoma tumor associated with a fulminant course of the disease has been encountered earlier (5, 6). Such a hemorrhage may cause massive catecholamine mobilization. Accordingly, noradrenaline treatment has rarely been successful. On the other hand full recovery has once been achieved with phentolamine (12).

Summary

A case of pheochromocytoma ending in a fatal hypotonic crisis is reported. The electrocardiogram taken at the beginning of the crisis, suggested the presence of acute anteroseptal infarction. Later large deformities of the ventricular complexes were found. The tumor contained only noradrenaline.

A comparison was made with two other cases of pheochromocytoma in which rather typical electrocardiographic changes were found.

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Follow up Studies of Patients with Superficial Gastritis and Patients with a Normal Gastric Mucosa

By

M. Siurala and Y. Vuorinen

The present authors have previously (18, 19) studied the development of gastric carcinoma and intrinsic factor deficiency in two groups of patients with chronic atrophic gastritis. In the present study patients with a normal gastric mucosa and patients with superficial gastritis were observed for approximately 8—10 years in order to study the occurrence of gastric carcinoma and the changes occurring in the anatomical and functional state of the gastric mucosa during the period of observation.

Material and methods

The series consisted of patients studied in 1952—53 at the Medical Outpatient Department, University of Helsinki and at the Hospital of Pohjois-Kymi, from whom 2—3 suction biopsy specimens were obtained and in whom no roentgenological nor gastroscopic signs of gastric carcinoma or gastric ulcer were encountered.

The series of superficial gastritis included patients, in whom, in addition to the above mentioned criteria, superficial edema and inflammatory cell infiltration together with well preserved body gland layer were encountered in the biopsy specimens. In addition, these changes were generally associated with prolongation and corkscrew-like appearance of the foveolae, and various alterations of the surface epithelium. The present cases of superficial gastritis corresponded well with the superficial gastritis of Schindler, the "Gastrite superficiale chronica" of Fieschi and Cheli (3) and the moderate or severe superficial gastritis of Joske et al. (8). The superficial gastritis group included 4 cases in which there were, in addition to signs of superficial inflammation, inflammatory cells scattered throughout the depth of the mucosa.

The normal gastric mucosa series was composed of patients in whom the biopsy specimens revealed a normal gastric mucosa (fig. 1). The patients with a normal mucosa fulfilled the criteria established by Palmer (11).

Our criteria as presented above were fulfilled by 93 patients with superficial gastritis and 168 patients with a normal gastric mucosa.

Of the 93 patients with superficial gastritis (including 4 cases with diffuse inflammatory cell infiltration — "interstitial gastritis") 43 were re-examined in 1961—62, 9 answered a questionary and 4 had died. No direct information was obtained from 37 patients.

Of the 168 patients with a normal gastric mucosa 49 were re-examined in 1961—62, 34 answered a questionary, 7 had died and no direct information was obtained from 78.

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Table I. Sex and age of patients with superficial gastritis and patients with a normal gastric mucosa

	Superficial gastritis					Normal gastric mucosa				
	Re-examined 1961–62	Answered questionary	Deceased	No direct information	Total	Re-examined	Answered questionary	Deceased	No direct information	Total
Males	20	3	2	15	40	25	20	7	43	95
Females	23	6	2	22	53	24	14	0	35	73
Mean age (years)	50.5	40.1	58.2	41.8	46.4	44.4	44.2	44.0	44.1	44.2
Range (years)	29–73	25–61	47–80	22–60	22–80	21–63	25–70	28–70	20–73	20–73
Total no. of cases	43	9	4	37	93	49	34	7	78	168

At time of first examination.

Table II. Male and female ratio, mean age and occurrence of gastric tumors in the different series

	Severe atrophic gastritis	Slight atrophic gastritis	Superficial gastritis	Normal gastric mucosa
Male	31	33	40	95
Femal	22	28	53	73
Mean ge (years)	50.8	52.0	46.4	44.2
F llow up period	5.8	6.0	8.4	9.5
Gastric carcinoma	5	1	—	—
Polyps	1	—	—	—

At time of first examinat on.

The fate of patients from whom no direct information was obtained was ascertained from death statistics and the cancer register. Since the Cancer Registry in Finland is informed of every diagnosed case of cancer, any diagnosed cases of gastric carcinoma could hardly be overlooked.

The following examinations were performed at the re-examinat on in 1961—62

1) X ray examination of the stomach

2) Histalog test meal, specimens were taken 30, 60 and 90 min. after administration of 5 mg of Histalog (Lilly) per 10 kg of body weight. In a part of the series, however, only one specimen was taken 60 min. after the injection of Histalog.

3) Gastric biopsy with the multipurpose suction biopsy tube.

In addition, the blood, urine and feces were examined.

In the superficial gastritis group 224 specimens were obtained from 93 patients at the first examination and 98 specimens from 40 patients at the second examination. The corresponding figures in the group with a normal gastric mucosa were 402 and 168, and 106 and 45, respectively. Thus 2—3 specimens per patient were obtained at each examination.

The follow up period averaged 8.4 years in the superficial gastritis group and 9.5 years in the group of normal gastric mucosa.

Results

Mean age, sex distribution and the occurrence of gastric tumors

The age and sex distribution in the two groups is given in Table I. In table II the same features and the occurrence of gastric tumors are compared with those in previously reported groups of atrophic gastritis (18, 19).

From table I it appears that in a considerable proportion of the present series no other information was obtained than that given by the death statistics and the cancer register. In the two groups of atrophic gastritis observed previously (18, 19) personal communication had

been attained with all or almost all patients. This may in part be due to the considerably shorter follow-up time than in the present series.

From table II it appears that in the atrophic gastritis groups there was a predominance of males, the ratios male/female being 1.41 and 1.25. In the superficial gastritis group, however, this ratio was 0.77. On the other hand, the ratio in the "normal" group was of the same order as in patients with atrophic gastritis.

Further, table II shows that in the atrophic gastritis groups the mean age at the time of the first examination was markedly higher than in the present groups. However, the observation period in the former groups was considerably shorter. Thus, at the time of re-examination the mean age of patients with severe atrophic gastritis was 56.6, with a less severe degree of atrophic gastritis 58.0, with superficial gastritis 54.8 and with a normal gastric mucosa 53.7 years. Hence at the time of re-examination there were no marked differences in the mean age of the groups.

As to the occurrence of gastric tumors, it is observed that gastric tumors had developed only in the groups with atrophic gastritis, mainly in the first reported group which included patients with a considerable loss of normal body glands and with metaplasia. However, in one patient with superficial gastritis the stomach wall showed slight rigidity with superficial peristalsis at the re-examination. The patient was achlorhydric and the biopsy specimens revealed slight atrophic gastritis with pseudopyloric metaplasia. The roentgenological appearance, however, was presumably due to vagotomy performed in 1951. The patient was asymptomatic, declined gastroscopy and has not been seen subsequently regardless of repeated inquiries.

Superficial gastritis group

The histological findings are based upon 224 specimens obtained from 93 patients at the first and upon 98 specimens from 40 patients at the second examination. The results of the re-examination differ markedly from those obtained 8—9 years previously. Atrophic gastritis (figs. 2 and 4) had developed in 20 patients, being of a rather severe degree in 7. Two patients showed a fairly normal gastric mucosa (fig. 6). On the other hand, in 18 patients the superficial alterations remained almost unchanged in spite of the long observation period.

Intestinal metaplasia was encountered at the re-examination in 5 patients (figs. 2 and 4), pseudopyloric metaplasia in 11 (fig. 2) and Brunner type of metaplasia in 2 (fig. 2). Of the 20 cases with atrophic gastritis at the second examination, the inflammatory signs were marked in 12 and less pronounced in 8. In the two cases in which an almost complete atrophy had developed the inflammatory changes were nearly absent.

In all of the 4 cases in which inflammatory cells were found scattered throughout the mucosa at the first examination severe atrophic changes had developed.

Considerable changes were also encountered in the patients' subjective complaints. Twenty three had postprandial distress at the first examination, but only 9 at the second. Six were without abdominal symptoms at the first and 16 at the second examination. Hence there was a tendency towards disappearance of abdominal distress, particularly of the

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Range (years)	29—73	25—61	47—80	22—60	22—80	21—63	25—70	28—70	20—73	20—7
Total no of cases	43	9	4	37	93	49	34	7	78	168

At time of first examination.

Table II Male and female ratio mean age and occurrence of gastric tumors in the different series

	Severe atrophic gastritis	Slight atrophic gastritis	Superficial gastritis	Normal gastric mucosa
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Mean ge (years)	50.8	52.0	46.4	44.2
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Gastric carcinoma	5	1	—	—
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In addition, the blood, urine and feces were examined.

In the superficial gastritis group 224 specimens were obtained from 93 patients at the first examination and 98 specimens from 40 patients at the second examination. The corresponding figures in the group with a normal gastric mucosa were 402 and 168, and 106 and 45 respectively Thus 2—3 specimens per patient were obtained at each examination.

The follow up period averaged 8 4 years in the superficial gastritis group and 9.5 years in the group of normal gastric mucosa.

Results

Mean age sex distribution and the occurrence of gastric tumors

The age and sex distribution in the two groups is given in Table I In table II the same features and the occurrence of gastric tumors are compared with those in previously reported groups of atrophic gastritis (18, 19)

From table I it appears that in a considerable proportion of the present series no other information was obtained than that given by the death statistics and the cancer register In the two groups of atrophic gastritis, observed previously (18 19) personal communication had

postprandial, during the period of observation. There was no correlation between the subjective condition and the changes in the gastric mucosa, since the latter showed an unmistakable tendency to progression.

Gastritis was the sole pathological finding in 18 patients at the first examination and in 4 patients at re-examination. Duodenal ulcer was present in one case at the first and in 4 cases at the second examination. Gastric ulcer had developed in 3 during the follow-up time however no active gastric ulcer was demonstrable at the second examination. The following groups were next in size functional gastro-intestinal disturbances (7 cases at the first and 13 at the second examination) various extra-abdominal diseases (5 at the first and 11 cases at the second examination) Hypochromic anaemia was uncommon (one case at the first and one at the second examination)

It appears that the HCl secretion remained unchanged in 21 was increased in 8 and decreased in 12 patients. The increase in HCl secretion occurred in two patients in whom the mucosa returned to normal and in 6 in whom the mucosal state remained unchanged. Of the 12 patients with a decrease in HCl secretion the gastritis progressed in 9 and remained unchanged in 3 patients.

The ordinary Schilling test was performed in the two patients in whom complete or almost complete atrophy had developed. It was *normal* (> 10 %) in one and a low normal (5—10) in the other case.

Patients with an originally normal gastric mucosa

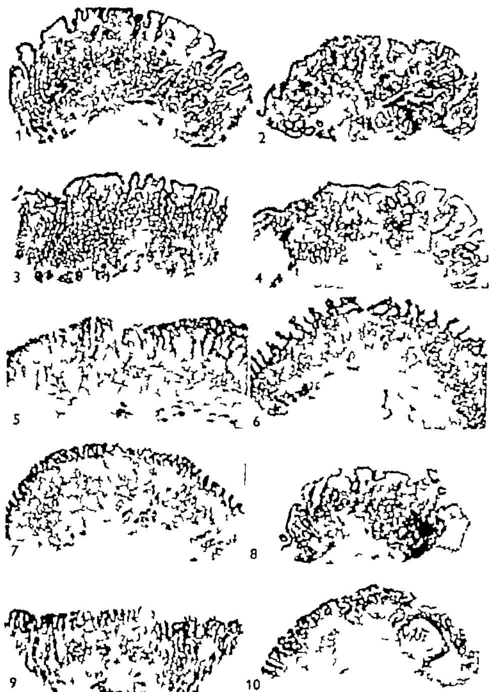
The results of the histological examination of 106 specimens obtained at the re-examination from 45 patients whose mucosa had been fairly normal 9—10 years ago, are as follows. In 29 cases the mucosa had remained normal. However superficial gastritis had developed in 12 (fig 10) and slight atrophic changes with occasional pseudopyloric metaplasia in 4 patients (fig 8) Intestinal tubules were not encountered in any specimen. Among the 12 cases with superficial gastritis there were 3 in which inflammatory cell infiltration extended from the surface to the muscularis mucosae.

The HCl secretion was unchanged in 16 increased in 3 and decreased in 18 patients. The decrease in secretion was in all but 5 patients associated with the development of gastritis.

Of the 16 patients in whom gastritis had developed 5 had been almost symptomless during the follow up period 3 had had duodenal ulcer 1 had developed mucosal prolapse into the duodenum, 1 hypochromic anaemia and 1 diabetes, and 4 had used considerable amounts of different analgesics because of rheumatoid arthritis and back pain. It is impossible to state whether these features bore any connection with the development of gastritis.

Eleven of the patients suffered from duodenal ulcer at the time of the first or the second examination or during the follow-up period. Gastric ulcer had not developed in any case. At the second examination gallbladder disease was encountered in 2 patients only hiatal hernia in 3 and mucosal prolapse in 1 The most common diagnoses at both examinations were various functional gastro-intestinal diseases or organic extra-abdominal diseases.

Definite hypochromic anaemia was encountered in 1 patient at the first and in an additional patient at the second examination.



Figs. 1—10 (H.E. magnific. × 25)
1 Male, 53. Biopsy specimen obtained in 1952. Marked superficial gastritis. *2* Same patient as in fig. 1. Specimen obtained in 1961. Severe atrophic gastritis. Intestinal glands in superficial layers and pyloric glands at the bottom (Brunner type of metaplasia). Marked inflammatory changes. *3* Female, 74. Specimen obtained in 1952. Distinct superficial gastritis. *4* Same patient as in fig. 3. Specimen obtained in 1962. Severe atrophic gastritis with intestinal metaplasia and moderate inflammatory signs. *5* Male, 41. Specimen obtained in 1952. Distinct superficial gastritis. *6* Same patient as in fig. 5. Specimen obtained in 1961. The mucosa appears fairly normal. *7* Female, 44. Specimen obtained in 1952. Normal gastric mucosa. *8* Same patient as in fig. 7. Specimen obtained in 1962. Severe signs of superficial inflammation and slight loss of normal body glands with pseudopyloric metaplasia. *9* Male, 45. Specimen obtained in 1952. Normal gastric mucosa. *10* Same patient as in fig. 9. Specimen obtained in 1962. Definite superficial gastritis with one large lymphoma.

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It appears that the HCl secretion remained unchanged in 21 was increased in 8 and decreased in 12 patients. The increase in HCl secretion occurred in two patients in whom the mucosa returned to normal and in 6 in whom the mucosal state remained unchanged. Of the 12 patients with a decrease in HCl secretion the gastritis progressed in 9 and remained unchanged in 3 patients.

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Patients with an originally normal gastric mucosa

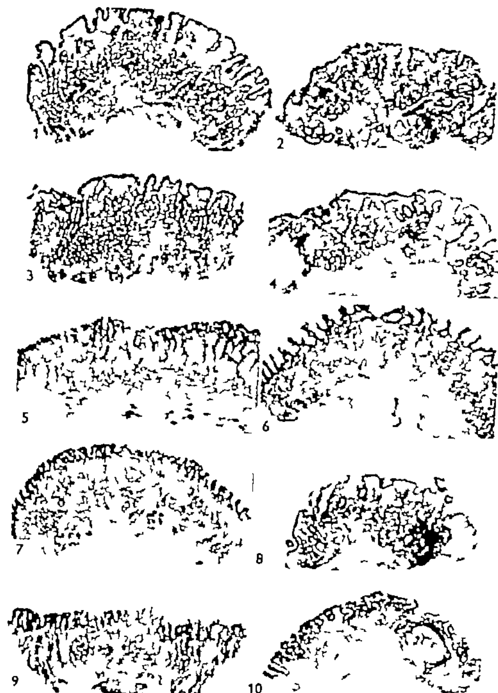
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In spite of the common development of gastritis, the subjective distress revealed the same tendency to disappear as in the patients with superficial gastritis. Nine were symptomless at the first and 17 at the second examination. Postprandial distress was present in 28 at the first and in 19 at the second examination.

Discussion

The present authors have so far had under observation during an average of 5.8—9.5 years four groups of patients in whom no roentgenological nor gastroscopic signs of gastric cancer or gastric ulcer were found at the first examination: 1) severe atrophic gastritis (53 cases), 2) atrophic gastritis of a less severe degree (63 cases), 3) superficial gastritis (93 cases) and 4) normal gastric mucosa (168 cases). The two latter groups served mainly as controls.

During the follow up period gastric carcinoma had developed in 5 patients and a benign tumor in 1 patient with severe atrophic gastritis. In all cases in this group some kind of metaplasia, particularly intestinal metaplasia, was encountered. In patients with atrophic gastritis of a less severe degree gastric carcinoma was encountered in one case, whereas none of the controls (with normal gastric mucosa or superficial gastritis) showed signs of gastric carcinoma at re-examination or had died of gastric carcinoma during the follow up period. Although there were some differences between the atrophic gastritis and control series in sex and age distribution of the patients, these differences were possibly insignificant, as discussed previously. Moreover, the control series were more than twice as large as the series with atrophic gastritis and the follow up period also was considerably longer. On the other hand, gastric carcinoma was in two cases of atrophic gastritis encountered fairly soon after the first examination. Hence, the possibility was taken into account that the tumor might have been present but had been overlooked at the first examination. However, it should be noted that the principles of selection and the examinations performed were the same in all the groups, and accordingly the chance of overlooking some gastric tumor at the first examination was the same in all groups. Moreover, the case history in these two cases suggested that atrophic gastritis had preceded the development of gastric carcinoma.

The Schilling test was performed in the majority of the re-examined patients with atrophic gastritis. In other groups it was performed only if severe atrophic changes had developed, as happened with two cases of superficial gastritis. Of 30 with severe atrophic gastritis the values were low in 4 (below 5 % of the dose) and a low normal in 5 patients (5—10 % of the dose). In the group with a less severe degree of atrophic gastritis there was only one patient with a low normal urinary radioactivity value. One of the 2 patients with superficial gastritis revealed a low normal and the other a normal urinary radioactivity value. These results agree well with those previously reported by Siurala et al. (16, 17) and Glass et al. (4). It should be noted that none of the patients with low values had developed clinical signs of vitamin B_{12} deficiency. As the progress of gastritis is apparently rather slow and furthermore, as the age of patients with atrophic gastritis is relatively high, it appears that the risk of developing manifest pernicious anemia is small, even for patients with severe atrophic gastritis. On the other hand, its

development is facilitated by an increased need for increased loss of, and a deficient supply of vitamin B_{12}. The nature of the gastric lesion leading to pernicious anemia is not yet clearly established. Since the importance of heredity in the development of pernicious anemia appears to be well documented (1 9 20) it is possible that this atrophic process might to some degree be an anomaly determined by heredity as has been suggested by Joske et al. (8) and Callender and Denborough (1) It should be noted, however that atrophic changes in the gastric mucosa are by no means the sole cause of an intrinsic factor deficiency leading to pernicious anemia. The reports of many authors suggest that deficiency of intrinsic factor particularly that occurring in members of families with pernicious anemia and in the pernicious anemia of children, may develop without the presence of atrophy of the gastric mucosa (2, 5 10, 12, 13 15)

At the re-examination of patients with superficial gastritis atrophic changes varying in kind and degree were encountered in half the cases. Normal gastric mucosa was observed in 2 patients only Of the patients with a normal gastric mucosa, superficial gastritis developed in 12 of the 45 cases, whereas atrophic changes of a slight degree were encountered in 4 patients only The results suggest that, once developed, superficial gastritis may persist or progress into atrophic gastritis. Accordingly the healing of gastritis would be uncommon. The results suggest further that atrophic gastritis develops by way of superficial gastritis, as has been assumed by many previous authors (6, 7 14) There are however two points that may make the validity of these results to some degree questionable, namely the patchy distribution of gastritis and the difficulty of obtaining specimens from the same areas at each examination. On the other hand it should be noted that 2—3 specimens per patient were obtained at each examination from different areas of the body of the stomach. Moreover the good correlation observed between the development of gastritis and the changes in HCl secretion may speak for the reliability of the results.

Summary

In order to study the occurrence of gastric tumors and changes in the state of the gastric mucosa, 93 patients with superficial gastritis and 168 patients with normal gastric mucosa, in whom no roentgenological nor gastroscopic signs of gastric tumor or gastric ulcer were present in 1952—53 were observed for average 8.4 and 9.5 years respectively Of the patients with superficial gastritis 43 were re-examined in 1961—62, 9 answered a questionary 4 had died during the follow-up period and no information was obtained on 37 patients except that received from the death statistics and the cancer register The corresponding figures in the normal gastric mucosa group were 49 34 7 and 78 respectively

Definite signs of gastric tumor were not encountered in any of these patients during the period of observation, whereas in previously reported series of atrophic gastritis, gastric tumor was detected in 7 of 116 patients.

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Brunner type of metaplasia in 2 patients. Complete or almost complete atrophy had developed in 2 patients The inflammatory changes were nearly absent in both. The Schilling test revealed a low normal value in one of them

In the series with normal gastric mucosa the mucosal state remained unchanged in 29 superficial gastritis had developed in 12 and a slight atrophic gastritis without metaplasia in 4 patients.

The secretion of hydrochloric acid showed a good correlation with the changes in the anatomical state of the gastric mucosa.

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Pancytopenia and Bone Marrow Hypoplasia in a Case of Paroxysmal Nocturnal Hemoglobinuria

By

Torolf Flatmark and Erik Myhre

Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon type of acquired hemolytic anemia in which the fundamental defect is intracellular. The anemia is often combined with granulocytopenia and thrombocytopenia, suggesting a defect also in the white cells and in the platelets. The etiology of the disease is unknown.

It is well known that patients suffering from PNH may develop a transitory crisis of regeneration failure (4) but chronic bone marrow hypoplasia or aplasia is very rare. A critical review of the literature reveals four cases of chronic hypoplasia (6, 18, 20, 26) and one case of chronic aplasia (25) in patients with PNH.

The purpose of this paper is to describe a case of PNH with bone marrow hypoplasia examined by modern hematological technic. The disease started as a hemolytic anemia with a hypercellular bone marrow; after a few months pancytopenia with bone marrow hypoplasia developed. Ferrokinetic studies in this latter stage suggested that a rapid destruction of young erythrocytes may in part explain the hypoplastic component of the anemia.

Submitted for publication June 20, 1962.

Case report

The patient was a 27 year-old Air Force pilot. The family history revealed no cases of anemia or jaundice. He was operated upon for an acute appendicitis in 1958; otherwise he had always been healthy.

Since May 1959 he periodically suffered from constipation and diffuse abdominal pains of moderate intensity. At the same time he became anemic and slightly jaundiced. He also observed slight bleeding from the gums when brushing the teeth. In June 1959 the hemoglobin was 7.4 g/100 ml and the serum bilirubin 1.6 mg/100 ml. The leukocyte count was 2,000/mm³ and the E. S. R. 50 mm in one hour. He was referred to hospital, where a macrocytic anemia was diagnosed. Treatment with parenteral liver, vitamin B_{12} and folic acid had no effect. In this period the hemolysis seemed to be moderately severe, and the bone marrow was hypercellular. The

Brunner type of metaplasia in 2 patients Complete or almost complete atrophy had developed in 2 patients. The inflammatory changes were nearly absent in both The Schilling test revealed a low normal value in one of them.

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ified serum from the patient were used. The erythrocyte acetylcholinesterase activity and the leukocyte alkaline phosphatase activity were both within the normal range.

Radioisotope studies were performed during a period of steady state, when the hemoglobin value was 7.0 g/100 ml (June 1961). The life span of the patient's erythrocytes was determined by tagging with Cr^{51} (23) and the ferrokinetics by Fe^{59} (15). The results are shown in fig. 2. The T/2 of the patient's Cr^{51}-labeled erythrocytes was 16 days, the mean life span of the erythrocytes thus being actually one-third of the normal. The daily hemoglobin breakdown was calculated to be 8.5 g (normal value 6—8 g). The disappearance of Fe^{59} from plasma was rapid, T/2 being 40 min. (our normal range is 74—145 min.). The serum iron concentration was 75 μg/100 ml, and the daily

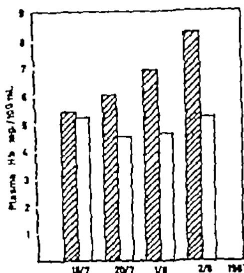


Fig. 1. Concentration of free hemoglobin in plasma. The shaded columns show the concentration at 8 a. m. and the open columns that at 6 p. m. No hemoglobinuria occurred.

Table II. The results of the laboratory tests diagnostic for PNH

Tests	The patient	Normal values	Method
Plasma hemoglobin (mg/100 ml)	5.8 (average) (fig. 1)	< 0.5	Harboe (13)
Hemoglobin binding capacity of the serum (mg/100 ml)	0	40—190	Nyman (27)
Hemoglobinuria	No	No	Gregersen (9)
Hemosiderinuria	Heavy	No	Rous (28)
Total iron excretion in the urine (mg/24 hours)	2.1 (average)	< 0.3	Bores (3)
Heat resistance test (hemoglobin concentration in serum supernatant after incubation for 6 hours) (mg/100 ml)	+ 4 C- 6.5 +20 C-15.0 +37° C-80.4	Negative	Hegglin & Maier (14)
Acid hemolysis test	++	Negative	Ham (10)
Acid hemolysis of thrombin-treated erythrocytes	++	Negative	Kirchmeyer (17)
Erythrocyte acetylcholinesterase (AChE) activity (micro31/min/1)	4.25	4.20 (average)	McOmer & Daniel (21)
Leukocyte alkaline phosphatase (LAP) activity (score)	62 (48)	44 (average)	Kaplow (16)

Table I Hematological findings

Date	Hemoglobin (g/100 ml)	Red cells (mill./mm³)	Reticulocytes		W.B.C./mm³	Granulocytes/mm³	Platelets/mm³	Serum iron (µg/100 ml)
			%	/mm³				
1 X. 1959	8.0	2.40	2.8	67,200	3,300	1 650	50,000	83
11 III 1960	9.6	3 40	1 4	47 600	5,800	1,500	41,000	168
10. VI 1960	6.5	2.50	0 7	17,500	4,500	1,395	50,000	255
7 I 1961	6.1	2.05	1.3	26,650	4,800	768	39,000	209
14 VII 1961	7.0	2.25	1 7	37,910	6,100	2,728	103 000	76
Normal range	14 0–18.0	4 6–6.2	0.5– 1 7	24 000–84 000	5 000–10 000	3,200–6,500	150,000–400 000	90-150

hemoglobin values dropped rapidly during intermittent febrile episodes.

The patient was admitted to this hospital October 1959 He was then anemic without visible jaundice The icterus index was 6 but the urine contained slightly increased amounts of urobilinogen The liver spleen and lymph nodes were not enlarged. The benzidine reaction in the urine and the stools was negative. The hematological findings are presented in table I Blood smears showed anisocytosis and some poikilocytosis of the red cells. There was a toxic granulation of the granulocytes, and a differential count showed 50 % granulocytes, 47 % lymphocytes and 3 % monocytes. The osmotic fragility of the red cells was normal. Coombs test was negative and cold antibodies were absent LE-cells (31) could not be demonstrated The bone marrow was hypercellular with a high proportion of normoblasts (the myeloid erythroid ratio was 1 1)

Large doses of steroids had no effect. In December 1959 splenectomy was performed The spleen weighed 440 g the histological examination showed only congestion. After the operation, a temporary rise of the platelets (maximum 342 000/mm³ on the 15th day) and the granulocytes (maximum 10,800/mm) was observed. Three months later however both thrombocytes and granulocytes had decreased to the pre-operative values. The operation had no effect on the anemia

Before operation the bone marrow was hypercellular with a high percentage of normoblasts, and four months after the operation it was markedly hypocellular Repeated aspirations from different sites during the last fifteen months always showed a hypocellular marrow with a relative increase of the lymphocytes the erythroid and myeloid series seemed to be equally reduced. On several occasions a few nucleated red cells were observed in the peripheral blood.

Results

Tests for PNH

The diagnosis of PNH was made on the observations presented in fig 1 and table II The plasma hemoglobin was significantly elevated and showed the typical nocturnal increase Hemoglobin could not be demonstrated in the urine, but considerable amounts of hemosiderin were found both extracellularly and within renal epithelial cells and granular casts. The urinary iron excretion was 2 18 and 2 02 mg per 24 hours on two consecutive days. The heat resistance test of the patient's erythrocytes was positive. The acid hemolysis test and acid hemolysis of thrombin[1] treated red cells were both strongly positive whether serum was from the patient or from a normal compatible donor Both tests were negative when normal erythrocytes and acid-

[1] Bovine thrombin, Topostasine Roche" was used.

However the term paroxysmal nocturnal hemoglobinuria is really a misnomer since the only constant findings in these patients are elevated plasma hemoglobin and hemosiderinuria (8, 11 19 29) If the concentration of free hemoglobin in plasma is low all the ultrafiltered hemoglobin may be reabsorbed in the kidneys. The reabsorbed hemoglobin is rapidly degraded to ferritin and hemosiderin, which may be demonstrated in the urine either extracellularly or within exfoliated tubular cells. As the paroxysmal nature of the disease is often not manifest, the diagnosis may be overlooked if the special tests for PNH are not performed. These tests seem to be rather specific for PNH, although a positive acid hemolysis test has been found in a case of Fanconi s familial aplastic anemia (5)

Our patient suffered from an acquired anemia which in the beginning was of a hemolytic type with a hypercellular bone marrow A few months after splenectomy a pancytopenia developed and the bone marrow was markedly hypocellular In this pancytopenic stage hemosiderinuria was the only clinical sign of increased hemolysis, in spite of the fact that the life span of his Cr^{51} labeled red cells was reduced to one-third of the normal. However due to the anemia the amount of hemoglobin actually broken down per day was only slightly increased. Therefore the common clinical signs of increased hemolysis were absent. The abnormal acid hemolysis of the patient red cells in normal, compatible serum, and the normal acid hemolysis of normal cells in his serum, showed an intracellular defect of his red cells. Further there were no abnormal erythrocyte antibodies in his serum. The presence of hemoglobinemia with a nocturnal increase, and of a marked hemosiderinuria suggested the diagnosis of PNH. As the heat resistance test, the acid hemolysis test and the acid hemolysis of thrombin-treated red cells all were clearly positive, the diagnosis of PNH seems to be proved

The erythrocyte acetylcholinesterase activity is commonly low in PNH. In our patient, however this enzyme activity was normal. Previously a normal acetylcholinesterase activity has been described in patients with moderate hemolysis (1 22) According to Tanaka et al. (30) the leukocyte alkaline phosphatase activity is low in PNH. However in two of seven patients they found normal values these patients were in remission In our patient also this enzyme activity was normal. Generally with a relatively small proportion of abnormal cells such enzyme defects may be impossible to demonstrate by assay of the whole blood cell population (22) Thus, the normal activity of these two enzymes may not disprove the diagnosis of PNH.

In our patient, pancytopenia and chronic bone marrow failure rather than hemolysis seemed to be the dominant signs. The disappearance rate of Fe^{59} from plasma was increased and the incorporation of Fe^{59} was rapid the first two days of the examination, this being in agreement with the diagnosis of hemolytic anemia. From the fourth day on, however the Fe^{59} content of the erythrocytes dropped from 48 to 38 per cent of the given dose. This could only mean that at least 20 per cent of the erythrocytes originally labeled with Fe^{59} had been destroyed during these nine days. Probably a considerably higher proportion of the Fe^{59}-labeled erythrocytes was destroyed, as most of the Fe^{59} liberated from destroyed red cells is reincorporated in new cells. Thus, it seems likely that hemolysis of young erythrocytes is an im-

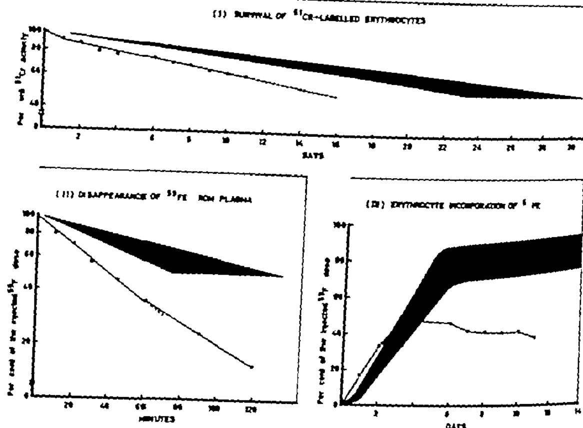


Fig 2 Radioisotope studies. The solid lines represent the values for the patient and the shaded areas the normal range.

plasma iron turnover was calculated to be 64 mg (twice the normal) The first two days, Fe^{59} was incorporated into the erythrocytes more rapidly than normal However the maximal incorporation was only 48 per cent of the given dose and the maximum was reached as early as 4 days the following 7 days the amount of Fe^{59} in the red cell mass decreased from 48 to 38 per cent. The daily effective hemoglobin production was calculated to 9 0 g The life span of Cr^{51} labeled platelets from a normal, compatible donor (2) was normal (10 days)

The patient required repeated blood transfusions to keep the hemoglobin concentration between 7 and 11 g/100 ml During the last two years he received 32 transfusions, each of 500 ml bank blood. As the patient observed dark urine after whole blood transfusions, saline-washed red cells were given At the end of this period a bone marrow aspiration revealed a very low content of hemosiderin (12)

Discussion

The clinical features of PNH are often bizarre and may be misleading particularly in cases with pancytopenia and signs of bone marrow failure Unfortunately the diagnosis of PNH is usually not considered in cases of hemolytic anemia if hemoglobinuria is not present

However the term paroxysmal nocturnal hemoglobinuria is really a misnomer since the only constant findings in these patients are elevated plasma hemoglobin and hemosiderinuria (8 11 19 29) If the concentration of free hemoglobin in plasma is low all the ultrafiltered hemoglobin may be reabsorbed in the kidneys. The reabsorbed hemoglobin is rapidly degraded to ferritin and hemosiderin which may be demonstrated in the urine either extracellularly or within exfoliated tubular cells. As the paroxysmal nature of the disease is often not manifest, the diagnosis may be overlooked if the special tests for PNH are not performed. These tests seem to be rather specific for PNH although a positive acid hemolysis test has been found in a case of Fanconi s familial plastic anemia (5)

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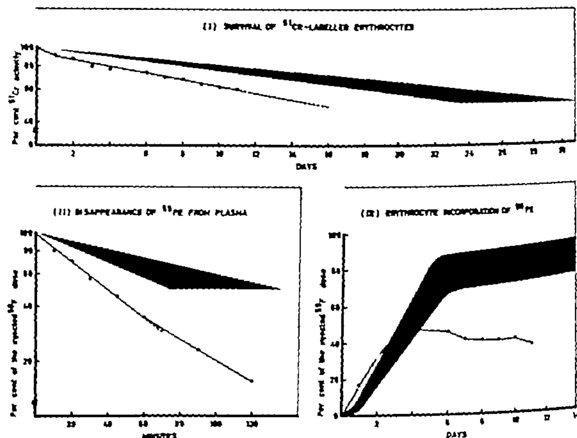


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portant factor in respect to the anemia. As the hemolysis of circulating reticulocytes in PNH is greater than that of the mature red cells (24) an increased hemolysis of the bone marrow reticulocytes seems possible. The discrepancy between the disappearance rate of Fe^{59} from plasma and the Fe^{59} incorporation in erythrocytes may be explained by destruction of erythroid cells within the bone marrow (7) In support of this view is the demonstration by Nussey and Dawson (26) of an increased autohemolysis of both erythroid and myeloid cells when the bone marrow from a patient with PNH was incubated *in vitro* However such an intramedullary cell destruction seems hardly to be the complete explanation of the bone marrow hypocellularity Therefore we have to conclude that in our patient the anemia may in part be due to an increased hemolysis of young red cells in the peripheral blood or within the bone marrow However the cause of the hypocellularity of the bone marrow in PNH is unknown

Another interesting finding in this patient is the absence of transfusional siderosis in spite of the transfusions of 16 000 ml bank blood during the last two years. By these transfusions he got a total of 7 g of iron. At the end of the observation period the serum iron concentration was 73 $\mu g/100$ ml and the bone marrow contained very little hemosiderin The observed iron loss in the urine could explain a loss of about 1 5 g during this time but probably the loss has been greater during periods of greater hemolysis. A very high urinary loss of iron in absence of overt hemoglobinuria has previously been reported (6 24) Therefore the risk of a transfusion siderosis in PNH patients seems to be negligible.

Summary

An atypical case of PNH is described. The disease started as a macrocytic anemia of a hemolytic type with a hypercellular bone marrow A few months after splenectomy pancytopenia developed and the bone marrow became hypocellular with marked reduction of both erythroid and myeloid cells. In this pancytopenic stage hemosiderinuria was the only clinical sign of increased hemolysis, though the life span of his Cr^{51} labeled red cells was reduced to one third of the normal. The disappearance rate of Fe^{59} from plasma was increased in spite of a nearly normal serum iron concentration, suggesting an increased erythropoietic activity of the bone marrow The incorporation of Fe^{59} into erythrocytes was increased the first two days, but later it dropped markedly indicating a rapid destruction of young red cells in the peripheral blood or within the bone marrow This destruction may in part explain the anemia and possibly the bone marrow hypocellularity in our patient.

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We wish to thank Dr R. Gulbrandsen, Rikshospitalet, for the estimation of leukocyte alkaline phosphatase activity and Dr O Lindgjerde Lier Hospital, for the determination of the erythrocyte acetylcholinesterase activity

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Distribution and Metabolism of Salicyl azo-sulfapyridine

I. A Study with C^{14}-salicyl-azo-sulfapyridine and C^{14}-5-amino-salicylic Acid[1]

By

Å. Hanngren, E. Hansson, N. Svartz and S. Ullberg

Salicyl-azo-sulfapyridine (salazopyrin® azulfidine®, in this paper abbreviated to SAP) results from diazotation of sulfapyridine and coupling of the diazonium salt with salicylic acid. Reductive splitting of the azo linkage in SAP yields sulfapyridine and 5-amino-salicylic acid (5-ASA).

It has been used in the treatment of ulcerative colitis since 1941 by Svartz (15, 17, 18, 20). Further Bargen (1, 2), Lagercrantz (8), Lennard-Jones et al. (9, 10), Watkinson (23) and others have published extensive experiences in ulcerative colitis. The drug is also used in regional enteritis (3), in certain forms of dermatoses (12, 14) and has also been tried in patients with rheumatoid arthritis (19). Clinical experience indicates a therapeutic effect but the mode of action of SAP is not completely known. The most immediate explanation is that of an antimicrobial action but the possibility that it acts partly or mainly on the metabolism of the connective tissue ought to be considered. Only a few details are known about its distribution pattern in the body. Affinity to elastin and collagen has been shown by fluorescence microscopy (6).

SAP is excreted into the urine only to small extent as unchanged drug (13, 16). Sulfapyridine and acetyl-sulfapyridine in about equal proportions have been found by chromatographic methods as metabolites (4). 5-ASA or its probable conjugates have not been demonstrated. Injected 5-ASA has been found by Helander (7) to have a strong affinity to connective tissue.

In our investigation we have compared SAP with its presumed metabolite 5-ASA as regards their distribution and metabolism. Both substances were labelled with

Submitted for publication June 20, 1962.

[1] A study of the distribution and metabolic fate of S^{35}-salicyl-azo-sulfapyridine and S^{35}-sulfapyridine will be published later.

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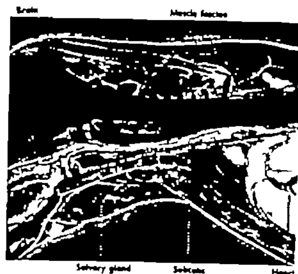


Fig 1 Autoradiogram showing the distribution of C^{14} salicyl-azo-sulfapyridine (C^{14}-SAP) 30 minutes after intravenous injection. Note high concentration in blood and connective tissues, the latter best seen between the lobes in e. g. the salivary gland and between the muscle bundles of the skeletal muscles.

C^{14} in the carboxyl group Whole body autoradiograms were made from sections of mice sacrificed at various intervals after intravenous and oral administration, and metabolic products in the urine liver and intestines were analyzed using paper chromatography

Methods

Preparation of C^{14} 5-amino-salicylic acid (11)

C^{14} salicylic acid, free from the para isomer was synthesized A coupling reaction between the C^{14}-salicylic acid and diazotized para nitroaniline was made. The resulting product was reduced to C^{14} 5-ASA. The specific activity calculated from the synthetical yields, was 18 μC/mg which was in agreement with values obtained from measurements in a gas flow counter

Preparation of C^{14} salicyl-azo-sulfapyridine (11)

C^{14}-salicylic acid was condensed with diazotized sulfapyridine in alkaline solution and the reaction product, precipitated by hydrochloric acid, was purified repeatedly by suspension in water (70°) Eventually the solid was dissolved in 2 M sodium hydroxide solution, boiled for 30 minutes and then precipitated by acid On the basis of the synthetical yield the specific activity was calculated to be 7 μC/mg

Upon analysis of similarly made non-radioactive SAP a 90—95 % assay was recorded. However when reduced, only 5-ASA was found and no 3- and 4-isomers.

Autoradiographic studies

Two series consisting each of 12 adult (20 g) white mice were injected intravenously One series was injected with 0.05 mg (corresponding to 0 9 μC) C^{14}-5-ASA per g body weight, and one series with 0 033 mg (corresponding to 0.23 μC) C^{14}-SAP per g body weight. The animals were anesthetized and killed by immersion in CO_2-acetone at the following times after injection 5, 15 and 30 minutes, 1 4 and 24 hours. SAP was also given by stomach tube in a dose of 0 033 mg per g body weight to three mice which were sacrificed 1 4 and 24 hours after the administration of the drug In two additional mice the common bile duct was ligated before the intravenous injection of C^{14} SAP and the mice killed 20 minutes and 1 hour after the administration.

The autoradiography was made according to methods previously described (21) The frozen animals were transferred to a refrigerated room (— 10° C) and mounted on large stages fitted to a sledge microtome. Sagittal 20 μ sections through the whole animal were taken and dehydrated at — 10° C.

Exposure was made by apposition against X ray film (Structurix, Gevaert) The exposure time was about 2 weeks for 5-ASA and about 4 weeks for SAP

Chromatographic studies

In order to study the metabolism of SAP and 5 ASA we have investigated the urine of mice during a 24-hour period.

The mice were injected intravenously with C^{14}-SAP or C^{14}-5-ASA and were kept in metabolic cages. The urine samples were taken at 1 4 and 24 hours after injection. The excretion of radioactivity was investigated in 4 mice injected intravenously with 5-ASA and 6 mice injected intravenously with C^{14} SAP Two mice received the compounds orally

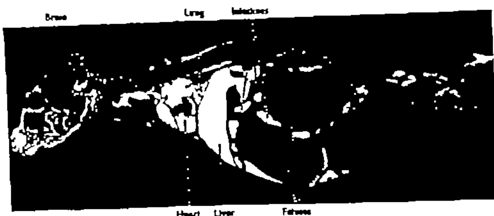


Fig. 2. Autoradiogram showing the distribution of C^{14}-SAP 30 minutes after intravenous injection. High concentrations are seen in the intestines. No activity can be traced in the fetuses and the central nervous system.

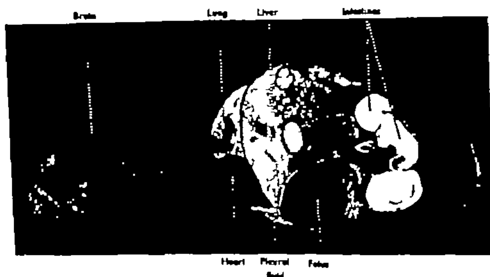


Fig. 3. Autoradiogram showing the distribution of C^{14} SAP 4 hours after intravenous injection. Note high concentration in the intestines, lung and pleural and peritoneal fluids. Very low activity is seen in the fetus.

In order to study the metabolic state of C^{14}-SAP in the intestine and liver, the whole intestine and the liver were removed from one mouse one hour after an intravenous injection and from one mouse 4 hours after oral administration.

The radioactive constituents in the urine and the tissues were separated by paper chromatography and detected by autoradiography of the chromatograms. The method was also used in the analysis of the radioactive 5-ASA and SAP before administration. The devel-

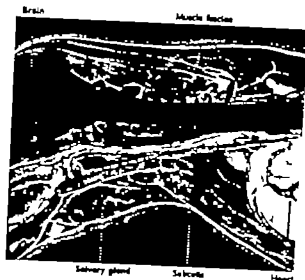


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C[14]-salicylic acid, free from the para isomer was synthesized A coupling reaction between the C[14]-salicylic acid and diazotized para nitroaniline was made. The resulting product was reduced to C[14] 5-ASA. The specific activity calculated from the synthetical yields, was 18 μC/mg which was in agreement with values obtained from measurements in a gas flow counter

Preparation of C[14] salicyl-azo-sulfapyridine (11)

C[14]-salicylic acid was condensed with diazotized sulfapyridine in alkaline solution and the reaction product precipitated by hydrochloric acid, was purified repeatedly by suspension in water (70°) Eventually the solid was dissolved in 2 M sodium hydroxide solution, boiled for 30 minutes and then precipitated by acid. On the basis of the synthetical yield the specific activity was calculated to be 7 μC/mg

Upon analysis of similarly made non-radioactive SAP a 90—95 % assay was recorded However when reduced, only 5-ASA was found and no 3- and 4-isomers.

Autoradiographic studies

Two series consisting each of 12 adult (20 g) white mice were injected intravenously One series was injected with 0 05 mg (corresponding to 0.9 μC) C[14] 5-ASA per g body weight, and one series with 0.033 mg (corresponding to 0.23 μC) C[14]-SAP per g body weight. The animals were anesthetized and killed by immersion in CO_2-acetone at the following times after injection 5, 15 and 30 minutes, 1 4 and 24 hours. SAP was also given by stomach tube in a dose of 0 033 mg per g body weight to three mice which were sacrificed 1 4 and 24 hours after the administration of the drug In two additional mice the common bile duct was ligated before the intravenous injection of C[14]-SAP and the mice killed 20 minutes and 1 hour after the administration.

The autoradiography was made according to methods previously described (21) The frozen animals were transferred to a refrigerated room (— 10° C) and mounted on large stages fitted to a sledge microtome. Sagittal 20 μ sections through the whole animal were taken and dehydrated at — 10° C.

Exposure was made by apposition against X-ray film (Structurix, Gevaert) The exposure time was about 2 weeks for 5-ASA and about 4 weeks for SAP

Chromatographic studies

In order to study the metabolism of SAP and 5 ASA we have investigated the urine of mice during a 24-hour period

The mice were injected intravenously with C[14]-SAP or C[14] 5-ASA and were kept in metabolic cages. The urine samples were taken at 1 4 and 24 hours after injection. The excretion of radioactivity was investigated in 4 mice injected intravenously with 5-ASA and 6 mice injected intravenously with C[14]-SAP Two mice received the compounds orally

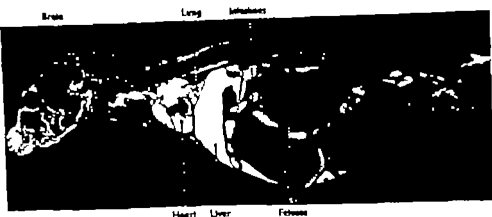


Fig. 2. Autoradiogram showing the distribution of C^{14}-SAP 30 minutes after intravenous injection. High concentrations are seen in the intestines. No activity can be traced in the fetuses and the central nervous system.

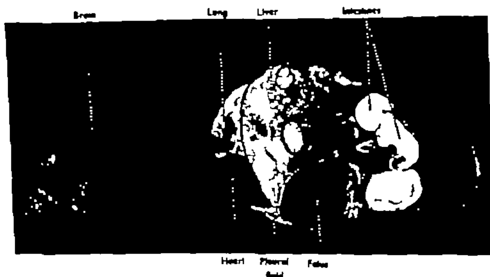


Fig. 3. Autoradiogram showing the distribution of C^{14}-SAP 4 hours after intravenous injection. Note high concentration in the intestines, lung and pleural and peritoneal fluids. Very low activity is seen in the fetus.

In order to study the metabolic state of C^{14}-SAP in the intestine and liver the whole intestine and the liver were removed from one mouse one hour after an intravenous injection and from one mouse 4 hours after oral administration.

The radioactive constituents in the urine and the tissues were separated by paper chromatography and detected by autoradiography of the chromatograms. The method was also used in the analysis of the radioactive 5-ASA and SAP before administration. The devel-

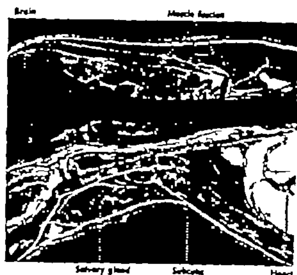


Fig. 1. Autoradiogram showing the distribution of C^{14}-salicyl-azo-sulfapyridine (C^{14}-SAP) 30 minutes after intravenous injection. Note high concentration in blood and connective tissues, the latter best seen between the lobes in e. g. the salivary gland and between the muscle bundles of the skeletal muscles.

C^{14} in the carboxyl group. Whole-body autoradiograms were made from sections of mice sacrificed at various intervals after intravenous and oral administration and metabolic products in the urine, liver and intestines were analyzed using paper chromatography.

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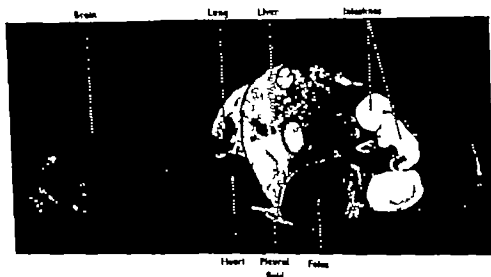


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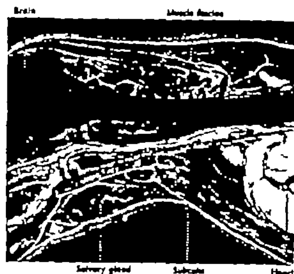


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Fig. 6. Autoradiogram showing the distribution of C^{14}-5-ASA 5 minutes after intravenous injection. High activity is seen in pleural and abdominal fluids. No activity in the fetus and nervous system.

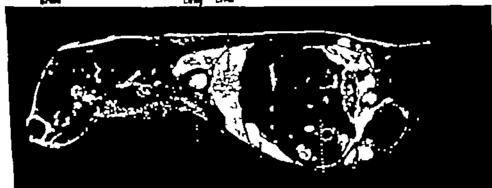


Fig. 7. Autoradiogram showing the distribution of C^{14}-5-ASA 1 hour after intravenous injection. Note high activity in the intestines, fetus, mammary glands, connective tissues.

Results

A. Tissue distribution

1. C^{14}-salicyl-azo-sulfapyridine (C^{14}-SAP)

The blood concentration of C^{14}-SAP keeps up at relatively high levels compared with those in most tissues. However already 5 minutes after injection a strong accumulation of the radioactivity in connective tissue is evident. This can be noticed e. g. in blood vessel walls, muscular fasciae, subcutaneous and submucous layers. The high radioactivity in connective tissue remains at 1 hour after



Fig 4 Detail of an autoradiogram showing the distribution of C^{14}-SAP 1 hour after injection. High activity is seen in the mucous membrane of uterus and vagina.

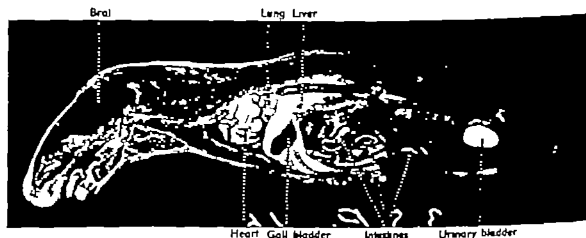


Fig 5 Autoradiogram showing the distribution of C^{14}-SAP 1 hour after intravenous injection. The common bile duct was ligated before injection. No activity is seen in the gall bladder High activity is seen in the intestines.

oping system was a mixture of pyridine isoamylalcohol and water (35 35 30 v/v) Whatman no 1 paper was used and the chromatograms developed by descending chromatography SAP and 5-ASA and their metabolites were then detected by ultraviolet light or color reactions using $FeCl_3$ and p-dimethylbenzaldehyde. Autoradiograms were prepared by pressing the chromatograms against X-ray (Industrex, Kodak) for appropiate periods of time. Quantitative analysis was made by cutting chromatograms in pieces and counting in a G M. tube.

Urine was chromatographed directly on paper and tissue after homogenization was first extracted with water at pH 8 and then with ethanol and the pooled samples were then put on the chromatographic paper



Fig. 9. Detail of an autoradiogram showing the distribution of C^{14} 5-ASA 1 hour after intravenous injection. High activity is seen in gall bladder and pleural fluid.

intestines, especially that of the colon, increases with time and dominates the picture after 4 hours (fig. 2 and 3).

An accumulation in the lung can be seen which is especially evident in the autoradiograms where the blood concentration has fallen (e. g. after 4 hours, fig. 3).

A high concentration of radioactivity was noticed in the peritoneal, pleural and synovial fluids. One hour after injection and later the concentration in these body fluids was significantly higher than in the blood (fig. 3 and 10).

A high level of activity was also noticed in the endometrium and in the vaginal secretion (fig. 4).

The penetration of C^{14}-SAP into the central nervous system seems to be blocked. Passage across the placenta also seems to be hindered as long as the SAP can be considered unsplit (fig. 2 and 3). Only

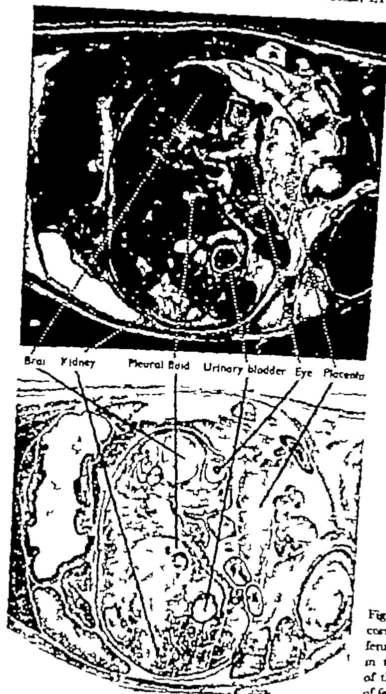


Fig 8. Detail of an utoradiogram and corresponding tissue section showing the fetus in fig 7 Note high concentration in the pleural fluid and aqueous humor of the fetus. No penetration to the brain of fetus.

injection but has disappeared after 4 hours (fig 1)

The liver rapidly achieves a slightly higher concentration than the blood The liver activity remains higher than the blood activity through the whole observation period but gradually decreases obviously mainly due to biliary excretion (fig 2 and 3)

A high activity appears in the mucosa and contents of the small intestine and colon in the animals whether or not the common bile duct is ligated (fig 3 and 5) In the unligated animals there was a high accumulation of activity in the bile while no noticeable activity was found in the bile of the ligated mice (fig 5)

The radioactivity in the lumen of the

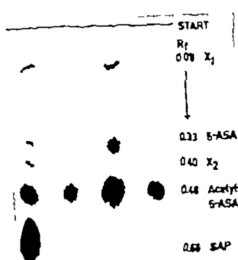


Fig. 11 Autoradiogram of paper chromatogram showing the metabolic stat of C^{14}-SAP and C^{14}-5-ASA in mouse urine after administration. Salicyl-azo-sulfapyridine 1—4 (A) and 4—24 (B) hours after the administration, 5-amino-salicylic acid 1—4 (C) and 4—24 (D) hours after administration.

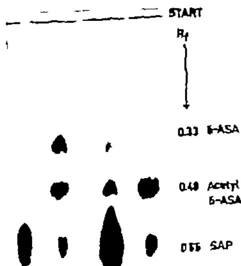


Fig. 12. Autoradiogram of paper chromatogram showing the metabolic state of C^{14}-SAP in the liver and the intestines. A = intestine and B = liver 1 hour after i. administration, C = intestine and D = liver 4 hours after oral administration.

Table 1 Qualitative analysis of different products found on paper chromatograms of urine and liver. Positive reaction to p-aminobenzaldehyde indicates free amino group and to ferric reagent free hydroxy group. Rechromatography of X X and acetyl 5-ASA after acid hydrolysis leave all radioactivity as 5-ASA

Reaction to	X Rf = 0.09	5-ASA Rf = 0.33	X_2 Rf = 0.40	Acetyl-5-ASA Rf = 0.48	SAP Rf = 0.65
p-aminobenzaldehyde (NH_2)	(+)	+	−	−	−
Ferric reagent (OH)	−	+	+	+	+
UV-light	+	−	+	+	−
Radioactivity	+	+	+	+	+



Fig 10 Detail of autoradiogram showing the hip joint. Note high activity in the synovial fluid. (C^{14}-SAP)

a very weak radioactivity can be seen in the fetuses 4 hours after injection. A strong accumulation is however noticed in the fetal membranes.

The concentration in the kidneys is high from 5 min to 1 hour after injection The level is highest in the pelvis.

The tissue distribution after oral administration was very similar to that after intravenous injection. The radioactivity concentration in the gastrointestinal lumen was, however considerably higher

2 *C^{14} 5-amino-salicylic acid (C^{14} 5-ASA)*

The blood concentration levels off somewhat faster for C^{14} 5-ASA than for C^{14}-SAP and the disappearance of tissue activity also proceeds slightly more rapidly

As for C^{14}-SAP a strong accumulation was observed in connective tissue from 5 minutes to 1 hour after injection (fig 6 and 7)

The accumulation in the liver of C^{14}-5-ASA is less pronounced than that of C^{14} SAP C^{14} 5-ASA rapidly accumulates in the bile (fig 9)

A similarly high concentration in peritoneal, pleural and synovial fluids was observed as for C^{14}-SAP and the changes of activity with time were similar (fig 9)

An accumulation of C^{14} 5-ASA was noticed in cartilage. The activity in the lung parenchyma did not exceed that of the blood

The penetration of 5-ASA and its metabolites into the central nervous system was blocked but the access to the fetus was apparently free. The distribution picture within the fetus was very similar to that of the dam (fig 8) No accumulation of radioactivity was observed in the fetal membranes.

B Metabolism

The chromatographic experiments on C^{14}-SAP indicated the formation of several radioactive products which were present in the urine and the investigated tissues following intravenous and oral administration (fig 11 and 12)

Put in order of increasing R_f-value, the following three radioactive spots could be identified 1) 5-ASA 2) acetyl-5-ASA and 3) SAP Two unidentified radioactive products were also seen one (Y_1) with a very low R_f value and one (Y_2) with its R_f-value between those of 5-ASA and acetyl 5-ASA. The data for the different products are given in table I

All five spots were seen on the chromatograms of the urine one and four hours after the administration (fig 11) Unchanged SAP represented the greatest part of the radioactivity at one hour whereas acetylated 5-ASA dominated in four hour urine (table II) The radioactivity of the urine collected 24 hours after injection seemed only to represent acetyl 5-ASA. The urine of mice given SAP orally was found to contain the same metabolites.

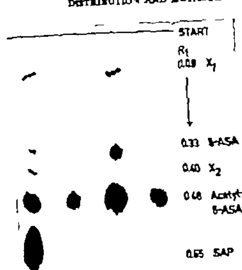


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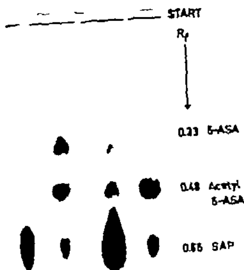


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UV-light	+	+	+	+	−
Radioactivity	+	+	+	+	+

Table II Semiquantitative values of different products found on paper chromatograms of urine and extracts from liver at different times after administration

Compound	R_f-value	Per cent in urine i.v. inject.		Per cent in urine oral adm. 4 hrs	Per cent in liver i.v. inj. 1 hr
		1 hr	4 hrs		
X_1	0.09	2	9	13	—
5-ASA	0.33	2	7	8	13
X	0.40	2	20	15	10
Acetyl 5-ASA	0.48	17	36	47	7
SAP	0.65	77	28	17	70

One hour after intravenous injection of C^{14}-SAP the intestinal radioactivity was exclusively in unchanged SAP and the liver was found to contain 5-ASA, acetyl 5-ASA and unchanged SAP (fig 12) Four hours after oral administration of C^{14} SAP mainly unchanged SAP some acetyl 5 ASA and traces of 5-ASA were found in the intestines, while the liver showed mainly acetyl 5 ASA and a smaller amount of SAP

Quantitative values for the different products are given in table II

Urine from mice given C^{14} 5-ASA showed three radioactive spots four hours and only one radioactive spot 24 hours after injection. Judging from the R_f-values the four hour urine contained unchanged C^{14} 5-ASA and acetyl C^{14} 5-ASA, and the unknown compound X_1 The 24 hour urine contained only acetyl C^{14} 5-ASA.

Discussion

Among the characteristic features of the distribution patterns of C^{14}-SAP may be mentioned the strong affinity to connective tissue and the high concentration in several body fluids such as peritoneal, pleural and synovial fluids. Such high concentrations in these body fluids are very rare at least judging from our experiences with a number of different substances investigated by this autoradiographic method (Ullberg (22)) To the extent that the radioactive compound in the C^{14} SAP autoradiograms represents or serves as a source for antibacterial activity the accumulation in these fluids may have therapeutic significance. This may also hold for the findings of a high concentration in endometrium and vaginal secretion.

The excretion of C^{14}-SAP via the gastro-intestinal wall may also be of interest in connection with its use in ulcerative colitis.

Generally a high activity was achieved in the gall bladder but ligation of the biliary duct — apparently due to a biliary stasis — prevented the labelled substance from reaching the gall bladder in observable amounts. This finding may explain negative results in the use of chemotherapeutics in clinical cases with biliary obstruction due to cholelithiasis.

Some similarities and some discrepances are found between the distribution patterns of C^{14}-SAP and C^{14}-5-ASA. Thus C^{14} 5-ASA also shows affinity to connective tissue and is found in high concentration in same body fluids as C^{14}-SAP Since salicylic acid has not so high an affinity to connective tissue (5) the

amino group seems to be of importance to direct the molecule (7)

Among the discrepancies it may be mentioned that the penetration to the fetus which is blocked for SAP is free for 5-ASA.

Other differences are that the SAP accumulation in the lung was not found for 5-ASA while specific concentration of 5-ASA is found in cartilage and mammae.

Since SAP is metabolized to some extent the radioactivity may also originate from some of its decomposition products. The metabolic studies, however show that C^{14}-SAP is the main radioactive *compound* during the first hour after injection. Later on metabolites dominate the picture. It is evident that the azo-molecule is split at the N = N linkage giving 5-ASA and sulfapyridine. The main radioactive compound excreted is not, however 5-ASA but presumably acetyl-5-ASA.

Summary

The autoradiographic distribution pattern after injection of C^{14}-salicyl-azo-sulfapyridine (C^{14}-SAP) labelled in the carboxyl group has been investigated in mice at various times after a single dose. A chromatographic separation of the radioactive compounds in urine, liver and intestines has also been made. Similar distribution and metabolism investigations have been performed by using part of the complex SAP molecule C^{14} 5-amino-salicylic acid. The metabolism investigation shows that injected SAP is split in the N = N-linkage giving sulfapyridine and 5-amino-salicylic acid. The radioactive compound excreted, however is mainly a metabolite of 5-ASA, probably acetyl-5-ASA. Among the characteristics in the autoradiographic pattern of C^{14}-SAP may be *mentioned* a pronounced binding to connective tissue and a *high concentration* in certain body fluids such as peritoneal pleural and synovial. Similar observations were made with 5-ASA. Injected C^{14}-SAP was also found to be secreted through the intestinal wall. A difference in distribution pattern is that while the penetration through the placenta of C^{14}-SAP was blocked C^{14} 5-ASA passed freely to the fetuses.

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Some similarities and some discrepancies are found between the distribution patterns of C^{14}-SAP and C^{14}-5-ASA. Thus C^{14}-5-ASA also shows affinity to connective tissue and is found in high concentration in same body fluids as C^{14} SAP Since salicylic acid has not so high an affinity to connective tissue (5) the

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From the Laboraty of Clinical Physiology Thoracic Clinics, Karolinska sjukhuset, Stockholm, Sweden

Systemic Arterial Pressure During Exercise in Patients with Pulmonary Hypertension

By

B. Jonsson and M. Lukianski[1]

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Direct measurement of arterial blood pressure during work has been made in patients with various kinds of heart disease. Usually these patients were studied in the supine position during right heart catheterization. In atrial septal defects pressure rise similar to that in normals was found (9) as in most cases of mitral stenosis (4 5 7). Bruce et al. (3) found a decrease in the arterial blood pressure in many cardiac patients studied in the upright position. These were cases with impaired myocardial function, arrhythmias and valvular stenosis. Cases with valvular incompetence on the other hand did not react with hypotension during exercise. The immediate effect on the arterial blood pressure during the first minute of work has, however not been reported in cardiac patients.

Some years ago we observed a case of primary pulmonary hypertension who showed a very marked and prolonged decrease in the arterial pressure on beginning work. This case suggested to us that study of the pressure reaction during work would be warranted in cases with high pulmonary vascular resistance.

Material

1. A control group of 10 healthy male volunteers (all included in studies of the normal circulation at rest and during exercise earlier reported (1 8)).

2. Three patients with primary pulmonary hypertension (cases no. 1—3). Congenital heart disease was excluded at autopsy in cases no. 1 and 2 and with angiocardiography in case no. 3. In cases no. 1 and 2 the foramen ovale was found to be closed.

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Stroke volume ml	Pressures, mm Hg									Pulm. vasc. resist. index
	Brachial artery			Pulmonary artery			PCV mean	Right ventricle		
	Systolic	Diastolic	Mean	Systolic	Diastolic	Mean		Systolic	End-diastolic	
64	120	73	89	63	32	41	9	63	9	10
48	140	82	103	114	55	79	10	114	30	
47	110	77	90	95	46	65	5	95	10	21
41	107	72	91	158	62	91	7	158	25	
79	120	67	86	94	37	53	9	72	6	18
68	125	77	96	119	37	86	7	119	16	
Pulm. circ.										
128	168	88	120	162	68	116	7	162	13	18
77	216	100	158	195	104	138	—	205	27	
37	134	66	90	86	52	—	11	—	—	19 7
—	152	83	114	128	81	97	—	—	—	
82	104	74	86	100	43	60	5	86	6	17
77	124	82	100	121	56	79	—	121	19	
32	118	70	86	39	22	31	24	38	3	4 4
31	122	68	86	65	23	48	35	64	7	
77	118	65	80	53	23	35	23	53	9	5.0
68	142	73	93	108	52	74	50	108	12	
54	158	88	112	73	37	50	27	—	—	7 4
37	166	95	116	110	52	75	58	74	7	
62	146	75	103	46	27	34	17	50	8	4.5
46	158	86	111	68	41	53	34	69	11	
51	138	81	101	122	63	91	33	122	7	17.3
41	150	82	105	161	79	114	35	161	13	

Methods

The methods and equipement used for right heart catheterization was the same as that practised in this laboratory and has previously been described (1). The arterial blood pressure was recorded through polyethylenecatheter introduced into the brachial artery by Seldinger' percutaneous technic (11). The tip of the catheter was advanced to the subclavian artery.

Table I Data obtained during heart catheterization

Case no.	Sex	Age years	Height cm	Weight kg	B.S.A. m	Blood volume l	Work load kpm/min	Heart rate beats/min	Oxygen uptake ml/min	Art. O_2-sat. %		Cardiac output l/min
Primary pulmonary hypertension												
1	♀	24	165	55 4	1 61	4.0	rest	75	238	95		4 8
							100	120	591	92		5.7
2	♀	21	165	49.5	1,55	3 7	rest	96	249	99		4.5
							150	132	646	92		5.3
3	♀	29	165.5	58 4	1 65	—	rest	54	215	98		4.5
							150	108	552	95		7.5
Patent ductus arteriosus												
										DA	BA	Pulm. circ.
4	♀	45	160	53 6	1.55	4 6	rest	80	316	88	97	9.6
							300	135	89	—	97	4.9
5	♀	40	163	59.3	1.64	4 6	rest	104	252	96	—	5.9
							300	142	856	—	92	—
6	♂	53	190	60 1	1.90	7.3	rest	66	259	—	91	5 4
							200	100	699	—	93	7 7
Mitral stenosis												
7	♂	31	176	64	1.82	—	rest	89	210	93		2.9
							150	137	559	96		4.2
8	♂	27	178	70.3	1.89	—	rest	60	244	97		4.6
							200	107	762	93		7.3
9	♀	47	157	55	1.54	4.8	rest	89	205	97		4 8
							300	178	920	95		6.5
10	♀	51	157	50	1 48	4 0	rest	91	228	95		5.6
							100	147	614	92		6.8
11	♂	41	163	62.5	1.67	—	rest	109	311	96		5 6
							100	151	743	96		6.2

PCV = pulmonary capillary venous DA = descending aorta BA = brachial artery

ance (cases no 4—6) The diagnosis was established by passage of the catheter from the pulmonary artery to the descending aorta.

4 Five cases with mitral stenosis and high pulmonary vascular resistance, e. g resistance units $\left(\frac{\text{pressure gradient, mm Hg}}{\text{cardiac output, l/min.}} \times \text{BSA}\right) < 4$ (cases no 7—11) Three of them (no. 7 9 and 10 had atrial fibrillation and the others sinus rhythm.

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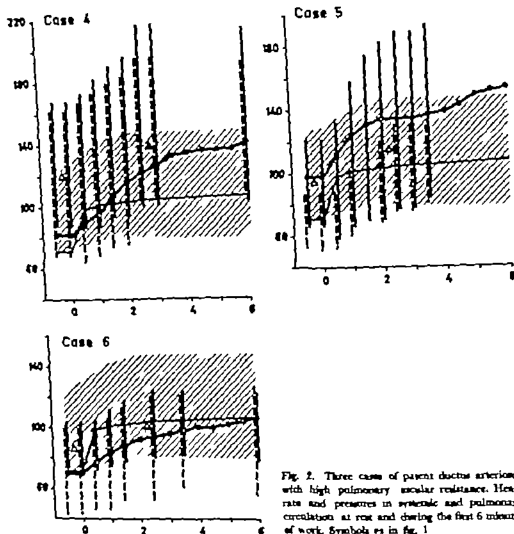


Fig. 2. Three cases of patent ductus arteriosus with high pulmonary vascular resistance. Heart rate and pressures in systemic and pulmonary circulation at rest and during the first 6 minutes of work. Symbols as in fig. 1

is shown in fig 1—3 For the normal cases only the a erage values are indica ted. In every normal case the pressure recorded 30 sec. after the start of work was higher than at rest. In each patient the pulse rate nd the pressures are indicated for each 30 sec. but in some cases mean pressure alone was recorded during periods of several minutes.

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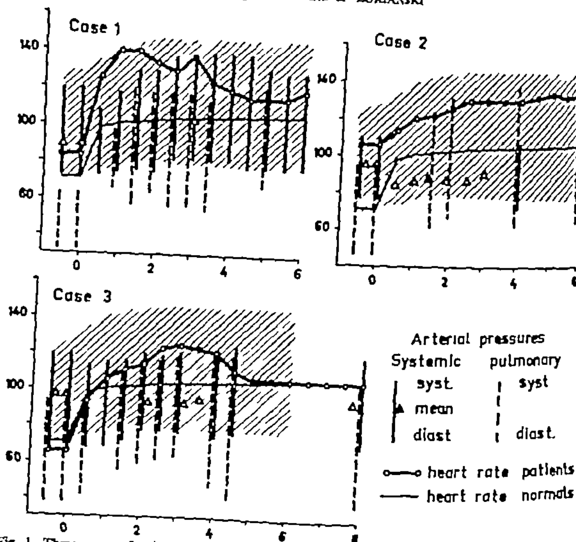


Fig 1 Three cases of primary pulmonary hypertension. Heart rate and pressures in systemic and pulmonary circulation at rest and during the first 6—8 min tes of work. y-axis = pressures in mm Hg and heart rate in beats per minut x axis = duration of work in minutes. The hatched area indicates th average variation of the systemic pulse pressure in the control group.

Procedure

After determination of the pressure and cardiac output on the patient at rest in supine position the exercise was performed in the supine position In some cases pressures and cardiac output were determined at several work loads, but as this paper deals solely with the effect of transition from rest to exercise only the data obtained during the first load are reported. The pressures were recorded during several minutes before exercise and while the feet were on the pedals and were continuously recorded during the first 3—4 minutes of work. The cardiac output according to Fick was determined during 5th—6th minutes of work and no pressures were recorded during this interval. As the pressures were recorded with a slow paper speed it was impossible to make planimetric calculations of the mean pressures. These were obtained by an electrical damping filter For that reason the mean pressure and the pulse amplitude could not be observed simultaneously

Results

Some anthropometric data and the main findings from right heart catheterization of the patients are summarized in table I. The effect on the arterial pressure during transition from rest to work

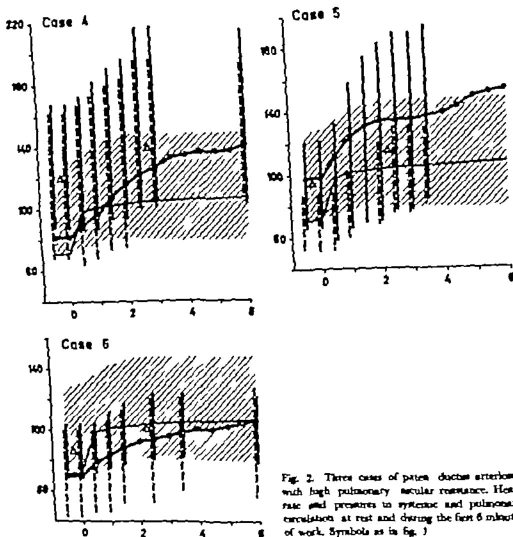


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In primary pulmonary hypertension (fig. 1) the arterial pressure decreased at beginning of work. In case no. 2 it was still low 6 minutes after the start of work

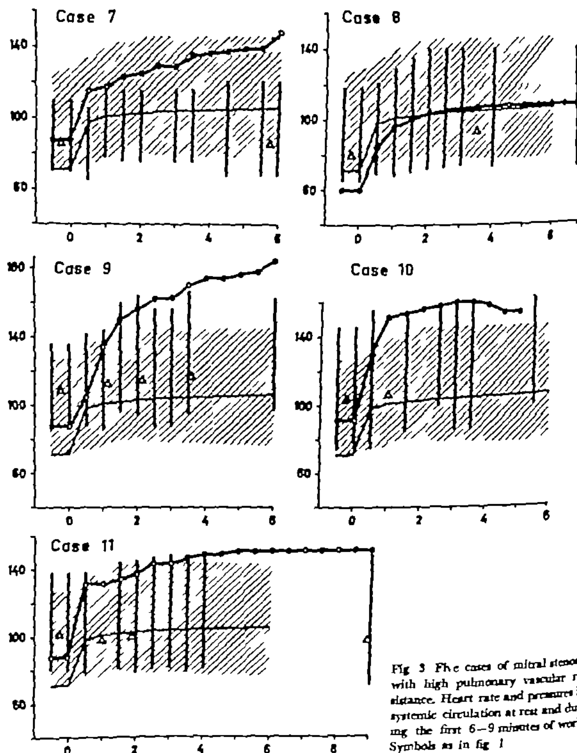


Fig 3 Five cases of mitral stenosis with high pulmonary vascular resistance. Heart rate and pressures in systemic circulation at rest and during the first 6–9 minutes of work. Symbols as in fig 1

but in cases no 1 and 3 it increased up to or above the resting level after 1 1/2—2 minutes of work. These two cases showed an unusual pulse reaction during work with an initial rise in rate followed by a decrease in rate after 3—4 minutes of work. The pressures in the pulmonary artery increased during exercise and did

not follow the pressures changes in the systemic arteries.

In the cases with patent ductus arteriosus the pulmonary vascular resistance was as high as in the cases with primary pulmonary hypertension. At the outset of work there was a continuous rise of the systemic pressure to a steady state level after 2—3 minutes of work. The pressure in the pulmonary artery increased in the same degree (fig 2)

Of the five cases with mitral stenosis and high pulmonary vascular resistance only one case (no. 7) showed a decrease of the systolic and diastolic systemic pressure during the first half minute f work. One minute after onset of work it had, however increased above the level at rest (fig. 3)

Discussion

In primary pulmonary hypertension a decrease of the systemic arterial pressure was observed during the first 2 minutes of work in the recumbent position. In normals there is, however a steady increase of the pressure during this period Cases with high pulmonary vascular resistance associated with a patent ductus arteriosus or mitral stenosis did not show a similar decrease of the systemic arterial pressure.

The size of the pulmonary blood volume is important for the left ventricular stroke volume (12) With a sudden increase in heart rate during transition from rest to work the pulmonary capacitance vessels will decrease in volume until the increased right ventricula output replaces the pulmonary blood volume This might explain the oscillations of the arterial pressure in normal individuals on beginning work in the sitting position, as described by Holmgren (6) As the pulmonary blood volume is smaller in the upright than in the supine position, the pressure fall at the beginning of work should be more marked in the upright position. This was also found by Holmgren (6) Our normal cases did not show any pressure fall at the beginning of work in the supine position. A pressure fall in the supine position is therefore a sign of disturbed circulation.

Normally there is only a slight increase in the pulmonary artery pressure even though the blood flow is markedly increased during exercise (1) This is due to the dilatation of the pulmonary vascular bed In primary pulmonary hypertension however there are anatomical lesions of small arteries with fixed resistance and the flow can increase only by the aid of increased pressure. An unchanged stroke volume at increased heart rate with shorter ejection time and increased pressure involve a considerable load on the right ventricle. In order for the right ventricle to maintain the stroke volume during exercise under these circumstances an increased diastolic pressure is required In all three cases studied there was a marked increase of the right ventricular end-diastolic pressure even during very slight work (see table I)

The marked and prolonged pressure fall t the beginning of work in cases with primary pulmonary hypertension can thus be explained by an abnormal distribution of the blood. During the first period of work the left ventricular stroke volume will be larger than the right, which will result in a shift of blood from the pulmonary to the systemic capacitance vessels. After a few beats this decrease of the pulmonary blood volume will result in a smaller stroke volume and decrease in systemic pressure.

The increased filling of the systemic veins will restore the right ventricular stroke volume resulting in a redistribution of the blood to the pulmonary veins and an increase in the left ventricular stroke volume and the systemic arterial pressure If the pulmonary blood volume is small at rest the above mentioned effect should be more marked The high pulmonary vascular resistance creates a barrier between the right ventricle and the pulmonary capacitance vessels which will result in a delayed refilling of these vessels after the beginning of work. In some cases the right ventricle might be incapable of compensating for this hindrance. In case no 1 which had the mildest pulmonary hypertension the pressure fall was of a rather short duration, but in case no 2 the pressure never increased after the initial fall to the level at rest.

Determination of the pulmonary blood volume at rest and during different stages of work as well as the estimation of the stroke volume, stroke by stroke, would be necessary in order to prove the validity of the explanation presented. Only the total blood volume was determined and it was within normal limits. In cases no 1 and 3 the pulse amplitude was measured during the first minute of work and was found to be small. After one minute of work it increased This may indicate a variation of the stroke volume.

In cases no 1 and 2 the arterial oxygen saturation was somewhat low during exercise, but as the foramen ovale was closed this could not be caused by a right to left shunt.

In cases with high pulmonary vascular resistance associated with a large ductus arteriosus the systemic arterial pressure increases normally during exercise. The wide communication between the pulmonary artery and the aorta will eradicate the pressure variations in the two systems. If the left ventricular stroke volume and the systemic pressure decrease, then the left to right shunt also decreases or the shunt may even be reversed and the systemic pressure will be restored. Therefore the oscillations in the systemic pressure seen at the beginning of work in primary pulmonary hypertension should not be present in cases with high pulmonary vascular resistance associated with a communication between the two arterial systems, as in patent ductus arteriosus and ventricular septal defect.

Two of the five cases with high pulmonary vascular resistance in association with a tight mitral stenosis showed a slight decrease in the arterial pressure the first minute of exercise but this was less marked and of shorter duration than in primary pulmonary hypertension. In tight mitral stenosis the pulmonary venous blood volume probably is increased and the stroke volume is small. As Holmgren (6) has pointed out the decrease of the arterial pressure at the beginning of exercise will be less marked if the pulmonary blood volume is large in relation to the stroke volume.

A decrease of the arterial pressure may occur in many diseases as a result of disturbed regulation of the cardiac output and peripheral resistance. This is seen in postural hypotension, also during work in the supine position (2) Patients with primary pulmonary hypertension seem to develop a marked exertional hypotension. They often have attacks of syncope and anginal pains and the incidence of sudden death is relatively high (10) The decrease of the arterial pressure which probably is more marked in the upright than in the supine position, may be an important factor for these attacks.

Summary

In primary pulmonary hypertension the systemic arterial pressure decreases during the first minute of work. This is not seen in patients with high pulmonary vascular resistance associated with a patent ductus arteriosus or mitral stenosis. The importance of the distribution of the blood volume for this pressure reaction is discussed.

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The increased filling of the systemic veins will restore the right ventricular stroke volume, resulting in a redistribution of the blood to the pulmonary veins and an increase in the left ventricular stroke volume and the systemic arterial pressure. If the pulmonary blood volume is small at rest the above mentioned effect should be more marked The high pulmonary vascular resistance creates a barrier between the right ventricle and the pulmonary capacitance vessels, which will result in a delayed refilling of these vessels after the beginning of work. In some cases the right ventricle might be incapable of compensating for this hindrance In case no 1 which had the mildest pulmonary hypertension, the pressure fall was of a rather short duration, but in case no 2 the pressure never increased after the initial fall, to the level at rest.

Determination of the pulmonary blood volume at rest and during different stages of work as well as the estimation of the stroke volume, stroke by stroke would be necessary in order to prove the validity of the explanation presented Only the total blood volume was determined and it was within normal limits In cases no 1 and 3 the pulse amplitude was measured during the first minute of work and was found to be small. After one minute of work it increased This may indicate a variation of the stroke volume

In cases no 1 and 2 the arterial oxygen saturation was somewhat low during exercise but as the foramen ovale was closed this could not be caused by a right to left shunt

In cases with high pulmonary vascular resistance associated with a large ductus arteriosus the systemic arterial pressure increases normally during exercise The wide communication between the pulmonary artery and the aorta will eradicate the pressure variations in the two systems. If the left ventricular stroke volume and the systemic pressure decrease, then the left to right shunt also decreases or the shunt may even be reversed and the systemic pressure will be restored. Therefore the oscillations in the systemic pressure seen at the beginning of work in primary pulmonary hypertension should not be present in cases with high pulmonary vascular resistance associated with a communication between the two arterial systems, as in patent ductus arteriosus and ventricular septal defect.

Two of the five cases with high pulmonary vascular resistance in association with a tight mitral stenosis showed a slight decrease in the arterial pressure the first minute of exercise but this was less marked and of shorter duration than in primary pulmonary hypertension. In tight mitral stenosis the pulmonary venous blood volume probably is increased and the stroke volume is small. As Holmgren (6) has pointed out the decrease of the arterial pressure at the beginning of exercise will be less marked if the pulmonary blood volume is large in relation to the stroke volume.

A decrease of the arterial pressure may occur in many diseases as a result of disturbed regulation of the cardiac output and peripheral resistance. This is seen in postural hypotension, also during work in the supine position (2) Patients with primary pulmonary hypertension seem to develop a marked exertional hypotension. They often have attacks of syncope and anginal pains and the incidence of sudden death is relatively high (10) The decrease of the arterial pressure, which probably is more marked in the upright than in the supine position, may be an important factor for these attacks.

Summary

In primary pulmonary hypertension the systemic arterial pressure decreases during the first minute of work. This is not seen in patients with high pulmonary vascular resistance associated with a patent ductus arteriosus or mitral stenosis. The importance of the distribution of the blood volume for this pressure reaction is discussed.

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Aus der medizinischen Universitätsklinik (Vorstand: E. Ask-Upmark, Dr. med.)
Uppsala, Schweden

Über eine an Leber's Opticusatrophie erinnernde heredodegenerative Krankheit

von

Ebba Exgoff

In einer im Jahre 1954 herausgegebenen umfassenden Monographie versucht Greenfield (11) unter dem Begriff der spinocerebellaren Degenerationen eine Gruppe progressiver Krankheiten die sich klinisch durch Koordinationsstörungen oder Ataxien und pathologisch-anatomisch durch Degeneration der verschiedenen Teile des Nervensystems, die für die Regelung unserer Bewegungen und ihr feineres Zusammenspiel verantwortlich sind, kennzeichnen. In dieser Krankheitsgruppe besteht eine Neigung zu entweder dominanter oder rezessiver Vererbung. Eine zentrale Stellung nehmen die hereditären Ataxien ein, die sich pathologisch-anatomisch durch degenerative Veränderungen des Sehnervens, des Kleinhirns, der Oliven und der langen kranialwärts und kaudalwärts verlaufenden Leitungsbahnen des Rückenmarks kennzeichnen. Sämtliche diese Teile des Nervensystems brauchen jedoch nicht auf einmal affiziert zu sein: man kann verschiedene Entartungskombinationen finden, die fliessende Übergänge zwischen den Krankheitsbildern, die wir von alters her als klassisch zu betrachten gewohnt sind, bedingen.

Bei der Redaktion am 21. Juni 1962 eingegangen.

In der medizinischen Klinik des Akademischen Krankenhauses in Uppsala sind während der letzten Jahre zum wiederholten Male eine Frau im mittleren Alter und der jüngste ihrer beiden Söhne gepflegt worden. Ihre Krankheitsbilder erinnern in vieler Hinsicht an die oben erwähnten heredodegenerativen Nervenkrankheiten.

Kasuistik

Fall I

Die Patientin ist eine geschiedene II-para, geboren 1910. Als Kind hatte sie Masern und Mumps. Während der ersten Schwangerschaft im Jahre 1929 bekam sie, zweieinhalb Wochen vor der Geburt, einen eklamptischen Anfall; die Entbindung war normal und erfolgte zur rechten Zeit. Die zweite Schwangerschaft im Jahre 1930 soll komplikationsfrei gewesen sein.

Die Patientin wurde im Jahre 1932 in der Ohrenklinik des Akademischen Krankenhauses zu Uppsala unter der Diagnose Otitis

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media subacuta sinistra behandelt. Sie teilte damals mit, dass das Sehvermögen während des letzten Jahres sich etwas verschlechtert hatte, und dass sie überdies beim Lesen und bei der Näharbeit von brennenden Augen beschwerden geplagt wurde. Bei der ophthalmologischen Untersuchung fand man, dass beide Papillen temporal deutlich abgeblasst waren, die Sehschärfe herabgesetzt war (V 0.5 sowohl des rechten als des linken Auges) und dass zentrale Farbenskotome vorhanden waren. Es wurde weiter festgestellt, dass die Patientin keine Ziffern lesen konnte, als sie mit Ishihara's pseudoisochromatischen Tafeln untersucht wurde.

Zwecks neurologischer Untersuchung wurde die Patientin in die medizinische Klinik übergeführt. Man fand ausser der vorher erwähnten bilateralen Atrophie des N. opticus, äusserst schwache linksseitige Bauchdeckenreflexe die leicht ermüdeten und sehr lebhafte Patellarreflexe beiderseits, am meisten jedoch links, wo man auch Patellarklonus fand. Die Achillessehnenreflexe waren lebhaft aber symmetrisch, und am linken Fuss lag ein angedeutetes Fächerphänomen vor. Der Allgemeinstatus und die routinemässigen Blut und Harnproben waren ohne Anmerkung ebenso der Liquorbefund nach Lumbalpunktion. WR in Blut und Liquor war negativ. Die Krankheit wurde damals als multiple Sklerose aufgefasst (und viermal mit Röntgen behandelt).

Im Jahre 1941 wurde die Patientin (und gleichzeitig mit ihr ihre beiden Söhne) in der Augenabteilung des Krankenhauses in Gävle untersucht man fand wie vorher bilaterale Atrophie des N. opticus, die Sehschärfe war aber etwas besser als früher (V > 0.7 an dem rechten Auge V 0.7 an dem linken) und die Gesichtsfelder für Weiss und Rot waren normal. Abgesehen von dem Augenbefund und einer unbeträchtlichen, linksseitigen Abnahme des Gehörs hinsichtlich der tiefen Töne (nach einer Otitis mit zurückgebliebener Perforation) war der Nervenstatus ohne Anmerkung auch die Röntgenuntersuchung des Schädels und der Sinus zeigte normale Verhältnisse. Bei der Röntgenuntersuchung der Augenhöhlen zeigte sich aber dass das rechtsseitige Foramen opticum kleiner als normal und nicht kreisrund, sondern oval mit horizontaler Längsachse war. Man war der Meinung, dass die Sehnervenatrophie ihren Grund in einem Entbindungstrauma habe (Zangengeburt).

Bei der Augenkontrolle in Uppsala im Jahre 1948 war die Sehschärfe auf beiden Augen V 0.5 und das Gesichtsfeld für Weiss ohne Anmerkung aber die Patientin konnte wie vorher keine von den Tafeln Ishihara's lesen. Beider Augenkontrolle, die dann in Gävle im Jahre 1954 vorgenommen wurde, war die Sehschärfe etwas verbessert (V < 0.7 an dem rechten, V 0.7 an dem linken Auge).

Abgesehen von der Resektion des Wurmfortsatzes im Jahre 1949 und von den oben geschilderten Symptomen war die Patientin im Grossen und Ganzen von 1932 bis Januar 1957 gesund. Von da ab begann sie im linken Oberarm von Muskelspannungen belästigt zu werden, die im Herbst 1957 in Zuckungen von ca. einer Minute Dauer übergingen. Von Dezember 1957 an hatte sie auch ein Schwächegefühl im linken Arm, im Juli 1958 traten Parästhesien im linken Arm hinzu und die Patientin fing ausserdem an, etwas ungeschickt zu werden.

Im August 1958 wurde die Patientin in die medizinische Klinik des Akademischen Krankenhauses in Uppsala zur Durchuntersuchung aufgenommen. Es zeigte sich dabei, abgesehen von einer Blutdruckerhöhung von 185/110 mm Hg ein normaler Allgemeinstatus. Neurologisch fand man Muskelzuckungen im linken Arm mit Streckung des Ellenbogengelenks, linksseitige Neigung zum Vorbeizeigen, Intentionstremor beim Fingernasenversuch und auf der linken Seite abgeschwächte Bauchdeckenreflexe. Wie früher fand man bilaterale Atrophie des N. opticus mit herabgesetzter Sehschärfe (beiderseits V 0.5) peripapilläre Atrophie und etwas verengte Retinalarterien und Zentralskotome. Bei der Lumbalpunktion fand man nichts Pathologisches. Der EEG-Befund war dagegen schwer pathologisch mit Deltafocus postzentral auf der rechten Seite. Rechtsseitige Carotisangiographie und Luftencephalographie waren ohne Anmerkung. Die routinemässigen Blut und Harnproben waren ohne Anmerkung ebenso die im Serum gefundenen Kalk und Phosphorwerte. Die Patientin wurde beurlaubt und bekam probeweise Tabl. Difhydan (0.1 g × 2).

Nach ungefähr sieben Wochen wurde die Patientin wieder in die medizinische Klinik

aufgenommen, weil sie immer noch an Zuckungen im linken Arm litt und ausserdem Schwindel und Schwächegefühl im linken Bein, wie auch Ausfall der linken Hälfte des Gesichtsfeldes auftrat. Bei der Aufnahme in die Klinik fand man Muskelzuckungen und Koordinationsstörungen des linken Arms wie im August 1958, Koordinationsstörungen des linken Beins, abgeschwächte Bauchdeckenreflexe links, lebhafte Patellarreflexe und eine Herabsetzung der groben Kraft im linken Arm und Bein. Ausser den oben geschilderten Augensymptomen fand man eine linksseitige homonyme Hemianopsie, die nach vierzehn Tagen teilweise zurückgegangen war. Während dieses Krankenhausaufenthaltes traten Zuckungen im linken Bein, das ausserdem immer mehr paretisch wurde und Neigung zu Spitzfuss-Stellung zeigte, auf. Die Patientin wurde psychisch labil und deprimiert. Wiederholtes EEG zeigte reichlich abnorme Aktivität vor allem in der rechten Parietalregion, aber auch eine deutliche Regression seit dem vorigen EEG im August 1958. Wiederholte rechtsseitige Carotisangiographie zeigte auch diesmal keine Zeichen pathologischer Veränderungen. Der Gehalt an Kupfer im Serum, der Oxydasewert und die Kupferausscheidung sowie die Ausscheidung von Aminosäuren im Harn und die Leberfunktionsproben waren normal. Die Patientin bekam diesmal physikalische Therapie und ausser Tabl. Dilhydan (0.1 g × 1) auch Tabl. Pargitan (5 mg × 2) und Tabl. Phenemal (0.1 g × 1).

Ende November 1958, drei Wochen nach der Krankenhausentlassung, war die Parese des linken Beins fast ganz zurückgegangen. Dies konnte auch bei erneutem Krankenhausaufenthalt im Januar 1959 festgestellt werden. Man fand jetzt, ausser den Symptomen, die bei dem kurz vorhergehenden Krankenhausaufenthalt vorhanden waren, lebhaftere Sehnen- und Periostreflexe des linken Arms und Beins und einen linksseitigen pos. Babinski. Der Zustand der Patientin während des folgenden Dreivierteljahres war erhältnismässig erträglich und sie konnte selbst den Haushalt führen, konnte jedoch keine Arbeit ausserhalb des Hauses finden, die sie auszuführen imstande war.

Die Patientin wurde im Oktober 1959 wieder in die medizinische Klinik aufgenommen, weil ihr Zustand sich während der letzten Wochen verschlimmert hatte und sie u. a. von Schwindel und schwankendem Gang belästigt wurde. Das objektive Symptomenbild war im Grossen und Ganzen unverändert, doch nahm die Einschränkung der Gesichtsfelder deutlich, wenn auch nur vorübergehend, zu. Während dieses Krankenhausaufenthaltes bekam die Patientin einen einzelnen, kurzdauernden, epileptiformen Anfall. Die Patientin wurde diesmal mit ACTH behandelt und nach einem guten Monat als beträchtlich gebessert aus dem Krankenhaus entlassen. Der Zustand ist danach ziemlich stationär geblieben.

Fall II

Der Patient, im Jahre 1930 geboren, ist der jüngste Sohn der Patientin, die im Vorausgehenden als Fall I geschildert worden ist. Er war während einer kurzen Periode, 1954—55, verheiratet und hat keine Kinder. Nach absolvierter Volksschule bildete er sich zum Elektriker aus und war zehn Jahre lang in einer Beleuchtungsfirma angestellt, musste aber wegen seiner Krankheit mit der Arbeit aufhören. In den letzten Jahren hat der Patient zeitweilig bei den Skutskärwerken im Lager gearbeitet. Anfang September 1960 wurde er krankgeschrieben und im November 1961 bekam er Invalidenpension.

Von den Kinderkrankheiten hat der Patient nur Masern gehabt. Im Jahre 1941, d. h. im selben Jahr als eine bilaterale Atrophie des N. opticus bei der Mutter und bei dem älteren der beiden Geschwister, einem Bruder, entdeckt worden war, soll der Patient in der Augenabteilung des Krankenhauses in Gävle untersucht worden sein. Er hatte damals keine subjektiven Augenbeschwerden, fing aber 1946 im Alter von *fünfzehn Jahren*, an, schlechter zu sehen. Die Herabsetzung des Sehvermögens nahm langsam zu. Bei einer Untersuchung im Frühjahr 1948 in der Augenpoliklinik des Akademischen Krankenhauses in Uppsala zeigte sich, dass er eine bilaterale Atrophie des N. opticus hatte und die pseudoisochromatischen Tafeln von Ishihara oder Boström-Kugelberg nicht lesen konnte. Die Sehschärfe sowohl des rechten wie des linken Auges war V. 0.5, die Gesichtsfelder für Weiss und Rot waren zu dieser Zeit ohne Anmerkung.

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aufgenommen, weil sie immer noch an Zuckungen im linken Arm litt und ausserdem Schwindel und Schwächegefühl im linken Bein, wie auch Ausfall der linken Hälfte des Gesichtsfeldes auftrat. Bei der Aufnahme in die Klinik fand man Muskelzuckungen und Koordinationsstörungen des linken Arms wie im August 1958, Koordinationsstörungen des linken Beins, abgeschwächte Bauchdeckenreflexe links, lebhaft Patellarreflexe und eine Herabsetzung der groben Kraft im linken Arm und Bein. Ausser den oben geschilderten Augensymptomen fand man eine linksseitige homonyme Hemianopsie, die nach vierzehn Tagen teilweise zurückgegangen war. Während dieses Krankenhausaufenthaltes traten Zuckungen im linken Bein, das ausserdem immer mehr paretisch wurde und Neigung zu Spitzfuss-Stellung zeigte, auf. Die Patientin wurde psychisch labil und deprimiert. Wiederholtes EEG zeigte reichlich abnorme Aktivität vor allem in der rechten Parietalregion aber auch eine deutliche Regression seit dem vorigen EEG im August 1958. Wiederholte rechtsseitige Carotisangiographie zeigte auch diesmal keine Zeichen pathologischer Veränderungen. Der Gehalt an Kupfer im Serum, der Oxydasewert und die Kupferausscheidung sowie die Ausscheidung von Aminosäuren im Harn und die Leberfunktionsproben waren normal. Die Patientin bekam diesmal physikalische Therapie und ausser Tabl. Difhydan (0,1 g × 1) auch Tabl. Pargitan (5 mg × 2) und Tabl. Phenemal (0,1 g × 1).

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Die Patientin wurde im Oktober 1959 wieder in die medizinische Klinik aufgenommen, weil ihr Zustand sich während der letzten Wochen verschlimmert hatt und sie u. a. von Schwindel und schwankendem Gang belästigt wurde. Das objektive Symptomenbild war im Grossen und Ganzen unverändert, doch nahm die Einschränkung der Gesichtsfelder deutlich, wenn auch nur vorübergehend, zu. Während dieses Krankenhausaufenthaltes bekam die Patientin einen einzelnen, kurzdauernden, epileptiformen Anfall. Die Patientin wurd diesmal mit ACTH behandelt und nach einem guten Monat als beträchtlich gebessert aus dem Krankenhaus entlassen. Der Zustand ist danach ziemlich stationär geblieben.

Fall II

Der Patient, im Jahre 1930 geboren, ist der jüngste Sohn der Patientin, die im Vorausgehenden als Fall I geschildert worden ist. Er war während einer kurzen Periode 1954—55 verheiratet und hat keine Kinder. Nach absolvierter Volksschule bildete er sich zum Elektriker aus und war zehn Jahre lang in einer Beleuchtungsfirma angestellt, musste aber wegen seiner Krankheit mit der Arbeit aufhören. In den letzten Jahren hat der Patient zeitweilig bei den Skutskärwerken im Lager gearbeitet. Anfang September 1960 wurde er krankgeschrieben und im November 1961 bekam er Invalidenpension.

Von den Kinderkrankheiten hat der Patient nur Masern gehabt. Im Jahre 1941, d. h. im selben Jahr als eine bilaterale Atrophie des N. opticus bei der Mutter und bei dem älteren der beiden Geschwister, einem Bruder, entdeckt worden war, soll der Patient in der Augenabteilung des Krankenhauses in Gävle untersucht worden sein. Er hatt damals keine subjektiven Augenbeschwerden, fing aber 1946, im Alter von fünfzehn Jahren, an, schlechter zu sehen. Die Herabsetzung des Sehvermögens nahm langsam zu. Bei einer Untersuchung im Frühjahr 1948 in der Augenpoliklinik des Akademischen Krankenhauses in Uppsala zeigte sich, dass er eine bilaterale Atrophie des N. opticus hatte und die pseudoisochromatischen Tafeln von Ishihara oder Boström-Kugelberg nicht lesen konnte. Die Sehschärfe sowohl des rechten wie des linken Auges war V 0,5, die Gesichtsfelder für Weiss und Rot waren zu dieser Zeit ohne Anmerkung.

media subacuta sinistra behandelt. Sie teilte damals mit, dass das Sehvermögen während des letzten Jahres sich etwas verschlechtert hatte, und dass sie überdies beim Lesen und bei der Näharbeit von brennenden Augen beschwerden geplagt wurde. Bei der ophthal mologischen Untersuchung fand man, dass beide Papillen temporal deutlich abgeblasst waren die Sehschärfe herabgesetzt war (V 0 5 sowohl des rechten als des linken Auges) und dass zentrale Farbenskotome vor handen waren. Es wurde weiter festgestellt, dass die Patientin keine Ziffern lesen konnte, als sie mit Ishihara s pseudoisochromatischen Tafeln untersucht wurde.

Zwecks neurologischer Untersuchung wurde die Patientin in die medizinische Klinik über geführt. Man fand ausser der vorher erwähn ten bilateralen Atrophie des N opticus, aus serst schwache linksseitige Bauchdeckenreflexe die leicht ermüdeten, und sehr lebhafte Patellarreflexe beiderseits, am meisten jedoch links, wo man auch Patellarklonus fand Die Achillessehnenreflexe waren lebhaft aber symmetrisch, und am linken Fuss lag ein angedeutetes Fächerphänomen vor Der Allgemeinstatus und die rutinemässigen Blut und Harnproben waren ohne Anmerkung, ebenso der Liquorbefund nach Lumbal punktion. WR in Blut und Liquor war negativ Die Krankheit wurde damals als multiple Sklerose aufgefasst (und viermal mit Röntgen behandelt)

Im Jahre 1941 wurde die Patientin (und gleichzeitig mit ihr ihre beiden Söhne) in der Augenabteilung des Krankenhauses in Gävle untersucht man fand wie vorher bilaterale Atrophie des N opticus, die Sehschärfe war aber etwas besser als früher (V > 0 7 an dem rechten Auge, V 0 7 an dem linken) und die Gesichtsfelder für Weiss und Rot waren normal Abgesehen von dem Augenbefund und einer unbe trächtlichen, linksseitigen Abnahme des Gehörs hinsichtlich der tiefen Töne (nach einer Otitis mit zurückgebliebener Per foration) war der Nervenstatus ohne An merkung auch die Röntgenuntersuchung des Schädels und der Sinus zeigt normale Verhältnisse. Bei der Röntgenuntersuchung der Augenhöhlen zeigte sich aber dass das rechtsseitige Foramen opticum kleiner als normal und nicht kreisrund, sondern oval mit horizontaler Längsachse war Man war der Meinung, dass die Sehnervenatrophie ihren Grund in einem Entbindungstrauma habe (Zangengeburt)

Bei der Augenkontrolle in Uppsala im Jahre 1948 war die Sehschärfe auf beiden Augen V 0.5 und das Gesichtsfeld für Weiss ohne Anmerkung, aber die Patientin konnte wie vorher keine von den Tafeln Ishihara's lesen. Beider Augenkontrolle, die dann in Gävle im Jahre 1954 vorgenommen wurde, war die Sehschärfe etwas verbessert (V < 0.7 an dem rechten, V 0 7 an dem linken Auge

Abgesehen von der Resektion des Wurmfortsatzes im Jahre 1949 und von den oben geschilderten Symptomen war die Patientin im Grossen und Ganzen von 1932 bis Januar 1957 gesund. Von da ab begann sie im linken Oberarm von Muskelspannungen belästigt zu werden, die im Herbst 1957 in Zuckungen von ca. einer Minute Dauer übergingen. Von Dezember 1957 an hatte sie auch ein Schwächegefühl im linken Arm, im Juli 1958 traten Parästhesien im linken Arm hinzu und die Patientin fing ausserdem an, etwas ungeschickt zu werden.

Im August 1958 wurde die Patientin in die medizinische Klinik des Akademischen Krankenhauses in Uppsala zur Durchunter suchung aufgenommen. Es zeigte sich dabei, abgesehen von einer Blutdruckerhöhung von 185/110 mm Hg ein normaler Allgemeinstatus. Neurologisch fand man Muskelzuckungen im linken Arm mit Streckung des Ellenbogengelenks, linksseitige Neigung zum Vorbeizeigen Intentionstremor beim Fingernasenversuch und auf der linken Seite abgeschwächte Bauchdeckenreflexe. Wie früher fand man bilaterale Atrophie des N opticus mit herabgesetzter Sehschärf (beiderseits V 0 5) peripapilläre Atrophie und etwas verengte Retinalarterien und Zentralskotome Bei der Lumbalpunktion fand man nichts Pathologisches. Der EEG-Befund war dagegen schwer pathologisch mit Deltafocus postzentral auf der rechten Seite. Rechtsseitige Carotisangiographie und Luftencephalographie waren ohne Anmerkung Die rutinemässigen Blut und Harnproben waren ohne Anmerkung ebenso die im Serum gefundenen Kalk und Phosphorwerte Die Patientin wurde beurlaubt und bekam probeweise Tabl. Difhydan (0 1 g × 2)

Nach ungefähr sieben Wochen wurde die Patientin wieder in die medizinische Klinik

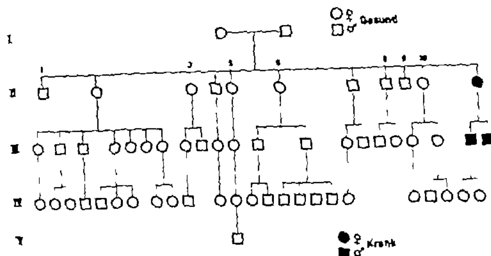


Fig. 1. Stammtafel der fünf untersuchten Generationen.

die durch zunehmende Beschwerden mit Krämpfen, schleudernden Zuckungen des linken Arms gekennzeichnet wurde; die grobe Kraft des linken Arms war unbedeutend erniedrigt. Es folgte dann eine verhältnismäßig lange Periode mit gebessertem Befinden, wonach der Zustand des Patienten sich im Herbst 1960 wieder verschlimmerte. Von dieser Zeit an ist er ununterbrochen arbeitsunfähig gewesen.

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Der Patient wurde im März 1961 zum Zweck einer Kontrolluntersuchung wieder in die medizinische Klinik aufgenommen ohne neue Symptome zu zeigen.

Fall III

Der Patient ist Chauffeur, im Jahre 1929 geboren, der ältere Sohn der als *Fall I* geschilderten Patientin. Der als Fall II geschilderte Patient ist sein jüngerer Bruder. Als Kind hat er an Enuresis nocturna gelitten, sonst war er im Grossen und Ganzen gesund.

Im Jahre 1941 wurde er in der Augenabteilung des Krankenhauses in Gävle, gleichzeitig mit der Mutter und dem jüngeren Bruder untersucht. Es wurde dabei festgestellt, dass auch er eine bilaterale Atrophie des N. opticus hatte; die Papillen, vor allem innerhalb der temporalen Hälfte, waren blass, die Retinalarterien etwas schmäler als normal, die Sehschärfe herabgesetzt (V. 0.5 am rechten Auge, <0.9 am linken); die Gesichtsfelder für Weiss und Rot normal. Abgesehen von dem Augenbefund war der Nervenstatus wie auch der Röntgenbefund des Schädels, der Augenhöhlen und der Sinus ohne Anmerkung. Bei wiederholter Augenuntersuchung 1946 war die Sehschärfe am rechten Auge V. 0.9—1.0, am linken V. 1.0; die Papillen waren blass, das Gesichtsfeld für Rot ohne Anmerkung und ohne Zentralskotom.

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Während einiger Monate Anfang 1959 war der Patient frei von Zuckungen. Im Herbst 1959 trat wieder eine Periode ein,

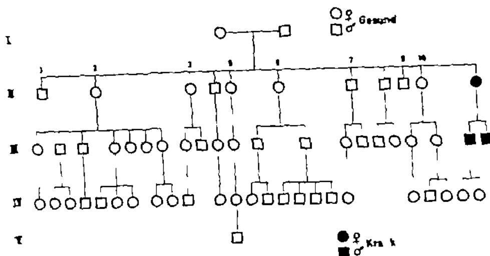


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nen Muskelkontraktionen spasmodischen Charakters, Muskelrigidität, Parästhesien, Opticusatrophie und Augenmuskelparesen kommen. Mehrere dieser Symptome sind bei Fall I und II zu finden.

Eine familiäre Krankheit mit Zügen von Olivopontocerebellärer Atrophie, Leber's Opticusatrophie und Friedreich's Ataxie (3 6 7 8, 9) wird von Woodworth, Beckett und Netsky (26) beschrieben. Eine Untersuchung von Schut (24) die 342 Personen von sechs Generationen aus demselben Geschlecht umfasst, lässt uns vermuten, dass verschiedene Ataxieformen einander sehr nahe stehen können. Er fand in 45 Fällen von Ataxie drei verschiedene Typen hereditäre Spinalataxie, hereditäre cerebelläre Ataxie und hereditäre spastische Paraplegie in vier von diesen 45 Fällen war eine Opticusatrophie vorhanden. Andere Augensymptome — wie Maculadegeneration — mit familiärem Auftreten zusammen mit Ataxie, sind auch beschrieben worden (4)

Von grossem Interesse in diesem Zusammenhang ist auch der von Ross (2, 17 23) hervorgehobene Umstand, dass gewisse Familien eine allgemeine genetische Vulnerabilität gewisser Teile des Nervensystems, die degenerieren und mehr oder minder komplizierte neurologische Störungen verursachen können, zu haben scheinen

Die drei Mitglieder der von mir geschilderten Familie scheinen an einer heredodegenerativen Krankheit zu leiden, die teils an Leber hereditäre Opticusatrophie, teils an Marie's hereditäre cerebellare Ataxie erinnert

Wünschenswert wäre, die biochemischen und genetischen (25) Grundlagen der Krankheit näher zu studieren. Diese Untersuchungen sind aber noch nicht ausgeführt worden.

Summary

A familial disorder with features of Leber's optic atrophy and Marie's hereditary cerebellar ataxia is described. In two members of a family a middle-aged woman and the younger of her two sons, the important clinical findings were bilateral optic nerve atrophy with central scotoma, impairment of visual acuity and color-blindness, incoordination, paresthesiae, partly hyperactive tendon reflexes, muscular spasms and depressive features. The son also suffered from major epilepsy The elder son had also a bilateral optic nerve atrophy but no symptoms of ataxia.

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Marie s hereditäre cerebelläre Ataxie (3 10 12 13 20 21 22) die im Alter von 20—30 Jahren zu beginnen pflegt, kennzeichnet sich durch lebhafte Sehnenreflexe, Inkoordination, Intentionstremor und skandierende Sprache. Hierzu kön-

nen Muskelkontraktionen spasmodischen Charakters, Muskelrigidität, Parästhesien, Opticusatrophie und Augenmuskelparesen kommen. Mehrere dieser Symptome sind bei Fall I und II zu finden.

Eine familiäre Krankheit mit Zügen von Olivopontocerebellärer Atrophie, Leber's Opticusatrophie und Friedreich's Ataxie (3, 6, 7, 8, 9) wird von Woodworth, Beckett und Netsky (26) beschrieben. Eine Untersuchung von Schut (24), die 342 Personen von sechs Generationen aus demselben Geschlecht umfasst, lässt uns vermuten, dass verschiedene Ataxieformen einander sehr nahe stehen können. Er fand in 45 Fällen von Ataxie drei verschiedene Typen: hereditäre Spinalataxie, hereditäre cerebelläre Ataxie und hereditäre spastische Paraplegie. In vier von diesen 45 Fällen war eine Opticusatrophie vorhanden. Andere Augensymptome — wie Maculadegeneration — mit familiärem Auftreten zusammen mit Ataxie, sind auch beschrieben worden (4).

Von grossem Interesse in diesem Zusammenhang ist auch der von Ross (2, 17, 23) hervorgehobene Umstand, dass gewisse Familien eine allgemeine genetische Vulnerabilität gewisser Teile des Nervensystems, die degenerieren und mehr oder minder komplizierte neurologische Störungen verursachen können, zu haben scheinen.

Die drei Mitglieder der von mir geschilderten Familie scheinen an einer heredodegenerativen Krankheit zu leiden, die teils an Leber's hereditäre Opticusatrophie, teils an Marie's hereditäre cerebelläre Ataxie erinnert.

Wünschenswert wäre, die biochemischen und genetischen (25) Grundlagen der Krankheit näher zu studieren. Diese Untersuchungen sind aber noch nicht ausgeführt worden.

Summary

A familial disorder with features of Leber's optic atrophy and Marie's hereditary cerebellar ataxia is described. In two members of a family, a middle-aged woman and the younger of her two sons, the important clinical findings were bilateral optic nerve atrophy with central scotoma, impairment of visual acuity and color-blindness, incoordination, paresthesiae, partly hyperactive tendon reflexes, muscular spasms and depressive features. The son also suffered from major epilepsy. The elder son had also a bilateral optic nerve atrophy but no symptoms of ataxia.

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From the Department of Medicine, the Department of Clinical Chemistry University Hospital of Lund, and the Department of Pharmacology University of Lund, Sweden

One-hour Subcutaneous ACTH Test with Determination of Plasma Corticosteroids

By

BENGT ARNER, PAVO HEDNER, TORD KARLEFORS and CLAUS RERUP

In practical endocrinology many methods for investigation of the ability of the adrenal cortex to respond to exogenously administered ACTH have been developed. ACTH has been given in different ways and its effect has been determined by several methods. One of the most employed ACTH tests is the 4-hour eosinophil count response to 25 IU of ACTH, injected intramuscularly described by Thorn (10). It was later modified by Jenkins et al. (6) who administered the ACTH as an 8-hour infusion.

When methods for determination of steroids in urine and blood became available these were soon adopted as parameters for assessment of the adrenocortical activity following administration of ACTH. These methods have the obvious advantage of being more specific than the eosinophil cell count. An example is the 4-hour plasma corticosteroid response to 25 IU ACTH injected intramuscularly (9).

In cases of relative adrenocortical insufficiency it is sometimes necessary to give ACTH as an intravenous infusion for several days to elicit an adrenocortical stimulation. Therefore the administration of ACTH in a single dose injected subcutaneously or intramuscularly is of diagnostic value only in case of a positive result, and must be limited to be used only as a screening test.

Screening tests of this type are, however, still of value in endocrinological practice. To increase the feasibility in ambulant practice we did some experiments to find out if the plasma corticosteroid concentration 1 hour after the subcutaneous injection of ACTH was already significantly increased above the pre-injection level. The investigation was performed in healthy subjects. In these experiments, eosinophil cells were also counted according to Thorn (10) to find whether the 1-hour plasma corticosteroid test might be a satisfactory alternative to the Thorn test.

Material and methods

The investigation included 33 healthy persons, 18 men and 15 women (medical students and nurses). The age ranged from 18 to 35 years. The subjects were kept fasting over night and during the test period, which was

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Table II Dat from tests with subcutaneous injection of 5 IU ACTH in 10 healthy subject

Subj. no.	Age	Sex	Eosinophil cells per mm³			Plasma corticosteroids, µg/100 ml		
			Pre-inject.	Post-inject.	% fall	Pre-inject.	Post inject.	Increase
1	26	♂	82	48	41	19.2	38.2	19.0
2	24	♂	86	30	65	23.4	37.2	13.8
3	22	♂	112	48	57	26.1	38.3	12.2
4	23	♂	126	62	51	14.9	32.0	17.1
5	33	♂	62	24	61	21.2	38.2	17.0
6	20	♂	30	10	67	24.6	37.4	12.8
7	22	♂	168	80	52	14.0	30.4	16.4
8	23	♂	232	96	59	18.4	39.8	21.4
9	23	♂	50	50	0	21.0	28.0	7.0
10	22	♂	172	92	47	19.9	31.0	11.1
		Mean	112	54	50	20.3	35.1	14.8
		Standard deviation	—	—	19	3.9	4.2	4.2
		Standard error of the mean	—	—	—	1.2	1.3	1.3

Results

Tables I nd II present the primary data from the ACTH tests performed, together with age and sex of the subjects.

In fig 1 the pre-injection values for the eosinophil cell counts has been plotted against the pre-injection values for the plasma corticosteroid concentrations. The eosinophil cell count revealed an uneven distribution not following the Gaussian curve Therefore no value for the standard deviation is given. The range was from 2 to 678 cells per mm Our mean pre-injection eosinophil count was 145 cells per mm³ The mean pre-injection plasma corticosteroid concentration was 21.0 per µg per 100 ml plasma, ranging from 12 to 32 µg per 100 ml with a standard deviation of 4.6 µg This is n accordance with values obtained earlier from healthy persons with the fluorimetric method in this laboratory No correlation was found between the pre-injection levels of plasma corticosteroids nd the eosinophil cell count as is also evident from fig 1

In fig. 2, the pre injection eosinophil cell count is compared with that obtained 4 hours after the injection of ACTH. The counts are given in absolute figures. The mean percentage fall was found to be 75 ± 17 % (S. D) after 25 IU of ACTH. 5 IU elicited an eosinophil cell fall of 50 ± 19 %.

In fig 2 the 45 degree line passing zero indicates the limit below which an eosinophil cell fall had occurred. All points in the figure lie below the 45 degree line but it is seen that some of them are situated quit close to t. It is also seen that in 17 out of 33 cases, the initial eosinophil count was less than 100 cells per mm According to Jenkins et al. (6) eosinophil cell changes measured in the range below this number are less accurate.

Fig 3 shows the relationship between the pre-injection level of plasma corticosteroids and the level 1 hour after the injection of 25 or 5 IU of ACTH. The mean plasma corticosteroid increase was

Table I Data from tests with subcutaneous injection of 25 IU ACTH in 23 healthy subjects

Subj. no.	Age	Sex	Eosinophil cells per mm³			Plasma steroids, µg/100 ml		
			Pre inject.	Post inject.	% fall	Pre inject.	Post inject.	Increase
1	23	♂	308	28	91	27 7	50.4	22.7
2	23	♂	2	0	100	25.2	35.2	10.0
3	21	♀	92	26	72	23.8	40.9	17.1
4	4	♂	270	58	79	18.2	40.9	22.7
5	21	♂	64	12	81	25.0	44.7	19.7
6	25	♂	30	18	40	21.9	36.8	14.9
7	22	♀	134	8	94	*0.7	32.2	11.5
8	20	♀	60	4	93	17.3	49.5	32.2
9	21	♀	140	18	87	21.9	50.6	28.7
10	22	♀	46	16	65	17.3	40.3	23.0
11	21	♀	12	4	67	20.9	45.0	24.1
12	21	♀	76	12	84	22.0	50.6	28.6
13	20	♀	90	32	64	25.2	51.6	26.4
14	21	♀	400	12	97	18.7	40.6	21.9
15	18	♀	74	38	49	15.9	36.3	20.4
16	31	♀	684	148	78	27.8	47.8	20.0
17	22	♀	184	18	90	30.8	44.0	13.2
18	19	♀	96	28	71	24.9	48.9	24.0
19	22	♂	370	152	59	26.5	49 4	22.9
20	30	♀	152	46	70	12.0	43.3	31.3
21	28	♀	60	10	83	20.7	43.7	23.0
22	24	♂	134	52	61	29 4	41 4	12.0
23	24	♂	188	104	45	19.8	29.6	9.8
Mean			159	37	75	22.3	43.3	21.0
Standard deviation			—	—	17	4.6	6.3	6 6
Standard error of the mean			—	—	3.5	1 0	1.3	1 4

started between 8 and 9 p. m and lasted for 4 hours. When samples for eosinophil cell count (capillary blood) and plasma corticosteroid determination (by venepuncture) had been drawn, 23 of the subjects were injected subcutaneously with 25 IU of ACTH and the remaining 10 subjects with 5 IU both doses per 60 kg body weight. One hour after the injection, blood was drawn by venepuncture for plasma corticosteroid analysis and 4 hours after the injection a capillary blood sample for eosinophil cell count was taken.

An oxycellulose-purified corticotrophin preparation (60 IU per mg) of a single batch was used for all injections.

Kindly supplied by Ferring A. B. Malmo, Sweden.

Th eosinophil cell counting was performed according to Rud (7) Jessen's counting chamber was used. One whole chamber was counted.

Blood samples for plasma corticosteroid determination were taken by venepuncture into tubes, prepared with a small amount of 5 % heparin solution. The analyses were performed according to a fluorimetric method described by Silber et al. (8) and Guillemin et al (4) The determinations were made on a 0 25 ml plasma sampl with synthetic hydrocortisone as the standard The result was expressed as µg of hydrocortisone per 100 ml plasma. In this laboratory the error of the method has been found to be 2.97 µg per 100 ml plasma (5)

venepuncture and injection did not contribute to the plasma corticosteroid increase following the injection of ACTH. The plasma corticosteroid decrease following saline may be due to the diurnal variation. The eosinophil count fell during 4 hours following the saline injection from 71 to 55 cells per mm³ (mean values). This fall may be explained by e. g. the spontaneous variation reported by Fisher and Fisher (1).

Discussion

In adrenocortical function tests, the eosinophil count changes following administration of ACTH have been widely used on account of the comparatively simple technic. There are however many factors that make the eosinophil cell response to ACTH uncertain. The wide range of the pre-injection eosinophil cell counts observed in fig. 1 is in agreement with the results of Foss Abrahamsen (2) from 84 healthy persons, the eosinophil cells being counted at the corresponding time of the day. According to Jenkins et al. (6) the significance of the eosinopenia following ACTH decreases with low initial counts. They excluded patients with less than 100 cells per mm³. We had in 17 out of 33 cases, initial counts below 100 cells per mm³ indicating that the eosinophil cell count quite often may lie in a range which has been recommended not to be used. Waldenström (11) demonstrated that the maximal decrease of the eosinophil cell count quite often occurred earlier or later than the 4-hour interval commonly used. A reliable registration of the maximal eosinophil decrease will therefore need at least three post-injection eosinophil counts, which diminishes the practicability of the method. In this investigation, only one post injection count was made according to Thorn (10). A factor of uncertainty in the eosinophil cell count as an index of adrenocortical activity is the considerable spontaneous variation reported by Fisher and Fisher (1). They demonstrated that in a material of healthy fasting subjects during 8—12 a. m. 10 % showed a spontaneous eosinophil decrease of 50 % or more, 45 % of the subjects showed a spontaneous fall of 25 %. Only 1/3 of the subjects had a stable or rising eosinophil cell count during the actual period. It is to be noted that the test period and the state of the subjects were the same in our experiments. We therefore feel uncertain how much of an observed eosinophil cell fall depended on a spontaneous change and how much really was due to an increased adrenocortical activity.

In this investigation, the eosinophil cell decrease following the injection of ACTH revealed a great dispersion when the absolute numbers were considered, partly due to the widely different initial counts. The percentage fall was, however, more constant with a decrease of 75 ± 17 S. D. % in the 25 IU group which is in accordance with the findings of Jenkins et al. (6). In the 5 IU group the standard deviation was 19 %. As the mean eosinophil decrease in this group was only 50 %, the 5 IU ACTH dose must be considered less suitable when the eosinophil cell count index is used. This view is partly based upon the spontaneous variation of the eosinophil count mentioned above.

Besides the great spontaneous variation of the eosinophils, it is also known that eosinopenia is not a specific index of the adrenocortical activity. Thus the administration of adrenaline has been shown to produce an eosinophil cell fall

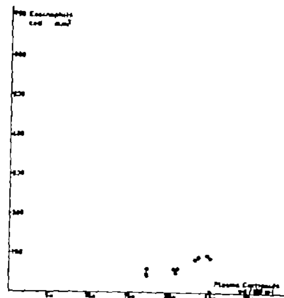


Fig 1 Relationship between pre injection values for eosinophil count (ordinate) and plasma corticosteroid concentration (abscissa)

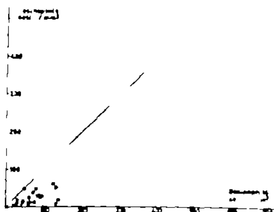


Fig 2 Eosinophil counts before (abscissa) and 4 hours after (ordinate) subcutaneous injection of ACTH into healthy subjects.
○ = 25 IU ACTH.
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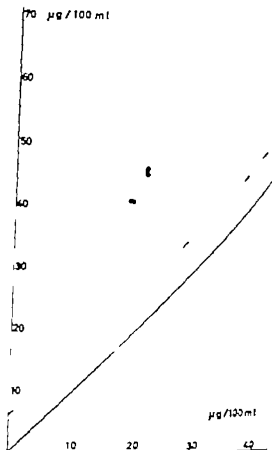


Fig 3 Plasma corticosteroid concentrations before (abscissa) and 1 hour after (ordinate) subcutaneous injection of ACTH into healthy subjects.
○ = 25 IU ACTH.
● = 5 IU ACTH.
Error of the method = 3.0 μg/100 ml.

21 ± 6 6 μg per 100 ml after 25 IU and 14 8 ± 4.2 μg per 100 ml after 5 IU of ACTH The difference between the increases for the two groups was significant at P < 0 01 Parallel with the 45 degree line passing through zero in fig 3 a line is drawn at a distance corresponding to double the error of the method (6 μg per 100 ml) It is seen that all values fall above this line, indicating a significant plasma corticosteroid increase at P < 0 05 for every single case. This was the case irrespective of whether the 25 IU or the 5 IU ACTH dose was given.

Four subjects were injected with 0.9 % NaCl solution instead of ACTH for control. All of them showed lower plasma corticosteroid concentrations 1 hour after the injection compared with the pre injection value. (Mean pre injection value 17 4 μg per 100 ml, mean post injection value 14 8 μg per 100 ml.) This result suggests that the trauma of

venepuncture and injection did not contribute to the plasma corticosteroid increase following the injection of ACTH. The plasma corticosteroid decrease following saline may be due to the diurnal variation. The eosinophil count fell during 4 hours following the saline injection from 71 to 55 cells per mm (mean values). This fall may be explained by e. g the spontaneous variation reported by Fisher and Fisher (1)

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without any effect on the plasma corticosteroid level (3)

The plasma corticosteroid concentration is known normally to follow a slightly decreasing curve from about 8 a.m. and during the day This is expected to have excluded falsely high post injection readings. Moreover the short test period (1 hour) gives little space for influence from spontaneous variation of the plasma corticosteroids.

The plasma corticosteroid determination in ACTH tests offers the obvious advantage of being more specific than the eosinophil cell count A direct replacement of the eosinophil count by measurements of plasma corticosteroids was made by Solem et al (9) who studied the 4-hour response to 25 IU ACTH injected intramuscularly As pointed out before such a method is principally a screening test. We therefore sought a modification to make it more convenient and less time-consuming The replacement of intramuscular injection of ACTH by subcutaneous injection does not seem to be any drawback regarding the effect of the injections.

In this series of experiments, the plasma corticoid response to ACTH was measured after 1 hour i. e before the maximal steroid increase had occurred In spite of this, a significant response of convincing size was obtained with 25 IU of ACTH With this method, a plasma corticosteroid increase of less than 10 μg per 100 ml should be suspected to be a sign of decreased adrenocortical capacity and the patient ought to be investigated more thoroughly

It is conceivable that an adrenocortical subcapacity manifests itself by a slow release of corticosteroids on ACTH stimulation. If so the damage might be revealed with greater accuracy with the 1 hour response than with the 4-hour response commonly used To our knowledge, however this possibility has not yet been investigated.

A significant plasma corticosteroid response was obtained in all subjects also with 5 IU of ACTH, as is evident from fig 3 With this dose, the lower 95 % increase limit was 6 3 μg per 100 ml plasma which is very close to twice the error of the method but yet an acceptable foundation for deciding whether an increase is probable or uncertain. The figures given above suggest that 5 IU probably is the lowest possible dose for a test of this type. It is possible that such a limit dose might reveal an adrenocortical capacity decrease better than a higher dose of ACTH

It is concluded that in ACTH tests the determination of plasma corticosteroids for several reasons is to be preferred to the eosinophil count index. The 1 hour plasma corticosteroid response to 25 IU ACTH proved to be useful as a screening test and may present theoretical advantages. With the plasma corticosteroid response 5 IU ACTH could be used but the eventual advantages of this dose must be further investigated before it can be used routinely

Summary

ACTH tests entailing measurement of the eosinophil cell decrease (Thorn test) were compared with the 1 hour plasma corticosteroid response to subcutaneous injection of 25 IU of ACTH. Under these conditions, the plasma corticosteroid index of adrenocortical activity proved to be useful as a screening test and ought to be preferred to the eosinophil cell count index for several reasons.

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Acta Medica Scandinavica. Vol. 173, fasc. 1 1963

From the Department of Clinical Physiology Karolinska Sjukhuset, Stockholm, Sweden

Circulation in the Calf at Rest, after Arterial Occlusion and after Exercise in Normal Subjects and in Patients with Intermittent Claudication

By

T. Strandell and J. Wahren

Obstructions of the main arteries to the lower limb are accompanied by more or less reduced oscillometric pulsations, with the result that pulsations and blood flow are sometimes used synonymously. But the collateral circulation which develops in these cases may under certain circumstances compensate for the reduced flow in the occluded vessel. Thus no typical changes in the quantity of the resting blood flow has been observed in patients with intermittent claudication (3, 8, 16).

With increased demand on the circulation one usually observes in these patients reduced ability to increase the blood flow, for example after exercise (8, 15, 17) or arterial occlusion (4, 5, 16). We have wished to illustrate these matters further and to investigate the correlation between the magnitude of the oscillometric pulsations at rest and the blood flow under different conditions.

Submitted for publication June 23, 1962.

Material

The material consists of groups N, P and P_r.

Group N is composed of 13 "normal subjects" (table I), seven of whom were male patients in the hospital with diagnoses unconnected with the heart or vascular system. The remaining six were healthy volunteers who had been subjected to a thorough clinical examination.

Group P consists of nine men with symptoms of intermittent claudication type in one leg (table I). The group includes a 27-year-old Egyptian with bullet wound affecting the external iliac artery. Arteriographic examination of this group revealed fairly well localized vascular obstructions. The subjective exercise tolerance in walking varied between 50 and 800 m.

Group P contains of six men with symptoms of intermittent claudication in both legs (table I). Arteriography showed in all cases widespread arteriosclerosis. The subjective exercise tolerance in walking was between 50 and 500 m.

Assisted by grant from Karolinska Institutet (reservationsanslaget), Stockholm.

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Submitted for publication June 25 1962.

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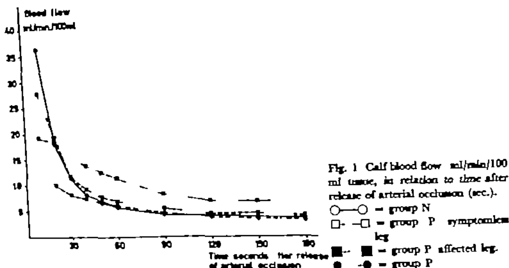


Fig. 1. Calf blood flow ml/min/100 ml tissue, in relation to time after release of arterial occlusion (sec.).
○—○ = group N
□- -□ = group P symptomless leg
■- -■ = group P affected leg.
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Reactive hyperaemia was induced by arterial occlusion at 200—225 mm Hg for 5 min. Single determinations of flow were made from 10 sec. up to 3 min. after the release of occlusion. These flow measurements were made on five subjects in group N, on all in group P and on five patients in group P_p.

Recording after exercise was done both with foot ergometer and with bicycle ergometer. The *foot ergometer* was so constructed that the subject, with straight knees, lifted 6 kg weight at intervals of one second in rhythm with a metronome by movements of the ankle via a lever system. The load (work intensity) was 40 kpm min. Tests with this ergometer were made on five patients in group P_p, working up to the threshold of pain with the affected leg and thereafter performing the same work with the symptomless leg. The blood flow was recorded from 30 sec. up to 10 min. after the end of work.

With the *bicycle ergometer* (7) the subjects were exercised in the prone position with one leg at a time. A metal spring was attached to the pedal to assist the return movement. The load was initially 100 kpm/min. and was increased every minute thereafter by a further 100 kpm/min. The final load was taken to be the last load at which the subject worked for 1 min. with a proportional increment of the completed part of the period at the next higher load. With five patients in group P the load was increased up to the threshold of pain for each leg. Five subjects in group N were then exercised at the same loads. Five other subjects in group N were exercised at the maximum load with each leg. The blood flow was recorded from 20 sec. up to 10 min. after the end of work.

Oscillography

For determination of the arterial pulsations in the lower legs oscillograms were recorded by the method of Gesenius & Keller (Boech & Speidel, Jungingen, Hohenzollern, W. Germany) on all individuals at rest in the prone position. The cuff was placed 8 cm below the knee joint. After reactive hyperaemia, i. e. about 15 sec. after 5-minute arterial occlusion, measurements were made on four patients from group P and on four from group P_p.

Results

Rest

The mean values of blood flow and arterial pulsations in the different groups are given in table II.

The resting blood flows are virtually identical in the different groups. The rather higher flow in the affected legs in

Table I Age height weight and blood pressure in the different groups. Mean standard deviation and standard error of the mean

No. of subjects	Age (years)	Height (cm)	Weight (kg)	Systolic B. P (mm Hg)	Diastolic B. P (mm Hg)
Group N					
13	59 1	175 7	76.1	136.2	80.4
	11 7	6.7	11.5	17.8	8.8
	3.3	1.9	3.2	4.9	2 4
Group P_1					
9	54 0	172.3	71.5	148.8	87.2
	11.9	7.6	9 3	22.2	19.3
	4.0	2.5	3.1	7 4	6 4
Group P_2					
6	60.2	173 6	68.7	144 1	85.0
	3.6	2.9	8.7	12.4	4 1
	1.5	1.2	3.6	5.1	1 7

N = normal subjects.
P_1 = patients with unilateral claudication.
P_2 = patients with bilateral claudication.

None of the patients had pain when at rest. No statistical difference was found between the mean resting blood pressures of the various groups.

Method

Plethysmography

The blood flow in the lower legs was measured with a thin walled rubber cuff the changes of volume of which were recorded under venous occlusion (3 6) This cuff was placed 8 cm below the knee joint A venous occluding cuff was placed above the knee and an arterial occluding cuff immediately below the volume recording cuff. The collecting pressure was 50 mm Hg (10 13) and the arterial occluding pressure 200—225 mm Hg

Recording was done in the prone position at rest, after reactive hyperaemia and after different forms of exercise

Table II Calf blood flow and arterial pulsations at rest and calf blood flow after arterial occlusion and after work in the different groups. Mean, standard deviation standard error of the mean and number of legs examined

	Group N	Group P_1 Symptomless leg	Group P_1 Affected leg	Group P
Blood flow at rest (ml/min/100 ml)	3.63	3.97	4.32	3.44
	1 18	2.40	1.57	1.23
	0.23	0.80	0.52	0.36
	26	9	9	12
Pulsations at rest (mm)	9 4	7.8	*2.2	1 6
	2.2	2.1	1.1	1.0
	0.4	0.7	0.4	0.3
	26	9	9	12
Blood flow 10 sec. after release of arterial occlusion	36.3	27 7	19 1	12.8
	7.2	7.9	7.9	6.3
	2.3	2.8	3	2.4
	10	8	7	8
Blood flow 20 sec. after maximal work	27	—	—	8.7
	4.8	—	—	5.3
	1 7	—	—	1.7
	8	—	—	10
Blood flow 20 sec. after submaximal work	19.5	—	—	—
	4.9	—	—	—
	1 6	—	—	—
	10	—	—	—

P < 0.05.
P < 0.001 where P indicates the probability that the difference from group N is caused by random factors. Other symbols as in table I.

Resting flow measurements were made in all cases and are given as the mean of ten inflow curves. The coefficient of variation for a single inflow curve was 12.5 % calculated on 155 duplicate determinations at rest in the group N The coefficient of variation for the mean of 10 inflow curves calculated from 26 duplicate series comprising 10 inflow curves including refitting of the cuff and recalibration of the pressure recording system was 15.2

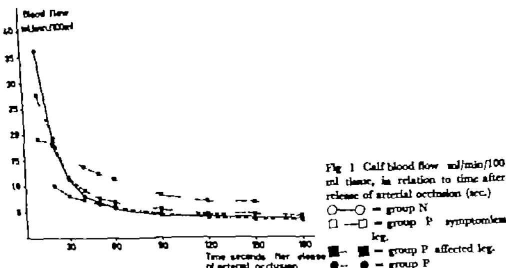


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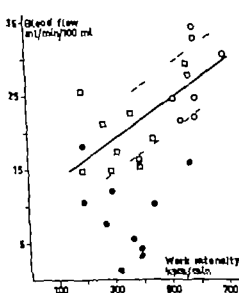


Fig. 4. Calf blood flow 20 sec. after bicycle work, ml/min/100 ml tissue, in relation to work intensity kpm/min. The straight line represents the regression line ($y = 0.0265x + 10.7$) obtained from the determinations in group N. The broken lines show the variation of ± standard error of estimate. Symbols as in fig. 3.

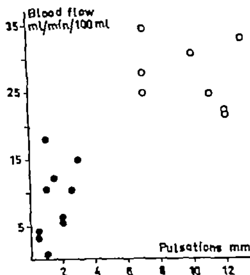


Fig. 5. Calf blood flow after maximal bicycle work, ml/min/100 ml tissue in relation to arterial calf pulsations at rest (mm). Symbols as in fig. 3.

min., average 2 min. 45 sec. No significant difference was found between the affected and unaffected legs for the same quantity of work, the mean values 30 sec. after the completion of exercise being 11.1 and 12.7 ml/min/100 ml respectively. This was probably because too small groups of muscles were engaged and because the measurements could not be made sufficiently quickly after the completion of exercise.

The blood flow after exercise on the bicycle ergometer is illustrated in figs. 3 and 4 and in table II. After maximal work (mean 350 kpm min.) the patients in group P had significantly lower flow (p 0.001) 20 sec. after the end of exercise than the volunteers in group N after the same work. In group N in which five individuals performed submaximal (mean 350 kpm/min.) and five others maximal work (mean 603 kpm/min.) there was a significant difference ($p < 0.001$) in the blood flow after the different intensities. Within group N the correlation between work intensity and blood flow 20 seconds after the end of work was significant ($r = 0.67 \pm 0.13$) as seen from fig. 4.

The relationship between the pulsations at rest and the blood flow 20 seconds after maximal work is statistically significant ($r = 0.82 \pm 0.08$). This is seen in fig. 5.

Discussion

The patients investigated were selected cases with typical symptoms of intermittent claudication. They can therefore not be said to be representative of individuals with low oscillometric calf pulsations. The resting blood pressures did not differ significantly in the various groups. The blood pressures immediately

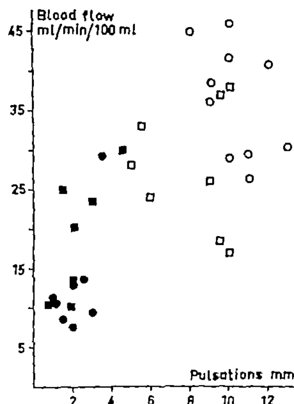


Fig 2 Calf blood flow 10 sec. after release of arterial occlusion, ml/min./100 ml tissue in relation to arterial calf pulsations at rest (mm) Symbols as in fig 1

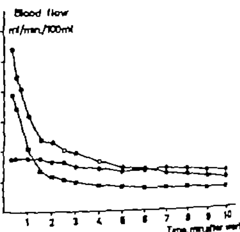


Fig 3. Calf blood flow ml/min./100 ml tissue, in relation to time after bicycle work, (min).
○—○ = group N maximal work.
□—□ = group N submaximal work.
●—● = group P_1, maximal work.

group P_1 is not significantly different from the flow recorded for group N ($p > 0.05$)

The pulsations in groups P and P are lower ($p < 0.001$) than in group N The unaffected legs in group P also have rather lower pulsations, but the difference is not significant ($p > 0.05$)

Reactive hyperaemia

The blood flow in the different groups after 5-minute arterial occlusion is shown in fig 1 and table II The mean value of the flow 10 sec. after the release of occlusion is lower ($p < 0.001$) for the affected legs in groups P_1 and P than for group N and probably lower ($p < 0.05$) for the symptomless legs in group P A probable difference ($p < 0.05$) existed between the affected and unaffected legs in group P_1 The higher flow 3 minutes after the release of occlusion in the symptomless legs in group P does not differ statistically from that of the other groups.

The relationship between the blood flow 10 sec. after arterial occlusion and the resting pulsations is significant ($r = 0.73 \pm 0.08$) This is seen from fig 2.

The arterial pulsations 15 sec. after 5-minute arterial occlusion were recorded in four cases in group P_2 and in four cases in group P No statistical difference was observed between these values and the pulsations at rest although the blood flow was more than doubled after occlusion. The pulsations before and after occlusion in the unaffected legs in group P_1 were 9.5 and 8.5 mm respectively in the affected legs 3.0 and 3.0 mm and in group P_2 1.8 and 1.7 mm.

Exercise

The blood flow after exercise with the foot ergometer was studied in group P The load was 49 kpm/min and the patients were able to exercise for 2—4

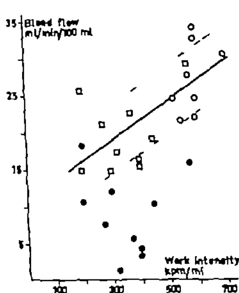


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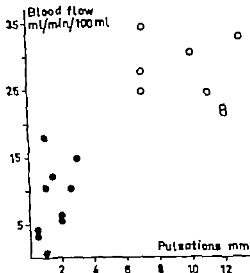


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after exercise were not measured but were probably essentially identical in the patient and normal groups, so that the differences in blood flow between the groups may be explained by differences in the peripheral resistance.

The mean calf blood flow at rest in the normal individuals was in close agreement with other reports in which water filled occlusion plethysmographs were used (1 2 16 17) and in which the means varied between 1 7 and 6 6 ml/min/100 ml tissue. The error of the method also appears to be of the same order of magnitude as in the determination of blood flow with water filled plethysmograph (2)

In patients with grave and widespread vascular lesions (group P_2) the same resting blood flows were measured as in normal individuals, whereas cases with more localized obstruction and greater possibility of developing collateral circulation (group P_1) exhibited rather higher flow values though not significantly different from the normal group Earlier studies have shown the same (8 17) lower (14 16) or higher (5) resting flows in cases with arterial occlusive diseases than in normal persons In the present study of ambulatory patients who have no pain when resting the resting blood flow is accordingly uncorrelated with the magnitude of the resting pulsations or with the severity of arterial vascular involvement judged from case history and arteriography There is such a relationship on the other hand between the amplitude of the resting pulsations and the severity of vascular involvement It must be pointed out that even in the symptomless legs in group P the resting pulsations are lower than the normal values for the corresponding age group (9)

The increase of blood flow after arterial occlusion in the normal cases was in conformity with earlier findings (11 13) In the patient groups the initial reactive hyperaemia was most reduced in group P_2, which contained the most widespread vascular lesions. In group P_1 the reactive hyperaemia was also reduced in the symptomless legs, which in combination with the slightly reduced resting pulsations suggests an incipient arterial obstruction In a study of patients with unilateral claudication, therefore, it may be inadvisable to use measurements on the unaffected leg as a check.

The blood flow process after exercise in the group P_1 patients showed marked variations compared with the normal flow curve. Thus the maximum flow occurred 5—10 min. after the end of exercise, a phenomenon which has been observed earlier (15) Conditions similar to those recorded for load and blood flow in the present investigation have been found in studies of the forearm (12).

The results presented are in close agreement with earlier reports of calf blood flow after arterial occlusion (4 5, 16) and after exercise (8, 15 17) in patients with arteriosclerosis obliterans. The blood flow after exercise and after arterial occlusion in the present material is correlated to the magnitude of the resting pulsations, but the scatter is fairly large This may be explained partly by the varying degree of collateral blood flow which does not contribute to resting pulsations to the same extent as the normal flow Oscillographic examination of patients with vascular occlusive diseases may thus provide some information about the degree of functional restriction, but not about the magnitude of the resting blood flow

Summary

1. The circulation in the lower legs of 9 men with unilateral (group P_1) and of 6 men with bilateral (group P_2) symptoms of intermittent claudication type, and of 13 individuals of similar age who were free from cardiac or vascular involvement (group N) was examined by determination of the arterial pulsations at rest and after 5-minute arterial occlusion, and by plethysmographic blood flow determination at rest, after 5-minute arterial occlusion and after leg exercise on a bicycle ergometer.

2. The affected legs exhibited low pulsations, normal resting blood flow and small increase of flow after arterial occlusion. After maximal leg exercise the blood flow was low compared with the flow after the same work performed by normal subjects, and still lower compared with the flow after maximal work by normal subjects.

3. In the normal group there was good correlation between load and blood flow immediately after exercise.

4. The resting blood flow was not correlated to the resting pulsations. The lowering of the resting pulsations, on the other hand, was well correlated to the lower increase of flow after arterial occlusion and after exercise.

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From the Department of Medicine II Sahlgren Hospital,
University of Göteborg, Göteborg, Sweden

A Clinical Study of a New Heparinoid

By

Kristoffer Korsan Bengtsen, Walter Berg and Gunnar Aspenström

Since the discovery and purification of heparin, many attempts have been made to produce synthetic substitutes. The goal has been to produce substances which have a lower price, are easier to administer and which have a longer duration of the anticoagulant activity. Heparinoids are chemical analogues of heparin, i. e. polysulphuric esters of polysaccharides. A few such compounds have been introduced into the field of medicine, but they have been abandoned because of their toxicity. A review of the problem of heparinoids has recently been published (8).

The purpose of this investigation was to study the medical usefulness of a new heparinoid compound synthetized by Geigy Switzerland. The substance is a calcium complex of sulphonated polygalacturonic acid methyl ester with an average molecular weight of 40 000.

The following was studied:

1. Effect of a single injection on haemostatic function.
2. Effect of repeated injections on haemostatic function.
3. Clinical effect of the therapy on a group of deep venous thromboses.
4. Immediate and late side reactions.
5. Occurrence of bleeding during the therapy.

In most of the patients who died during or after the treatment, autopsy was performed.

In patients who were treated for more than 3 days — and in some of the others — daily examinations of the white cells, haemoglobin concentration, urinary protein, and urinary sediment were made. Benzidine tests were made on every stool; plasma-creatinine was analyzed twice during the treatment.

Material

1. The effect of a single injection of heparinoid was studied in 12 healthy young, male, medical students.

2. All patients who had deep venous leg thrombosis, pulmonary embolism, and myocardial infarction and who were admitted to the hospital during the period of the investigation were included in the study. The material consisted of three groups:

1) 15 patients with venous thrombosis.

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b) 10 patients with pulmonary embolism or venous thrombosis together with a complicating disease (the patients in group a) and b) were treated with heparinoid for 7 days)

c) 76 patients most of whom had myocardial infarctions (the patients in this group were treated with heparinoid during the first 3 days of treatment with dicumarol)

3 Consecutive cases were picked from the hospital record and were used as control groups for the side effects. The control groups consisted of

a) 55 patients, who had been treated with heparin (125 mg administered subcutaneously twice daily for 1—3 days at the start of dicumarol therapy)

b) 37 patients who were on long term treatment with dicumarol and who had never received heparin

Methods

Bleeding time was determined by a modification of Ivy s method (5) The upper normal limit is 11 minutes.

Capillary fragility was determined by applying suctions cups (diameter 22 mm) to the supra spinatus regions with a negative pressure of 200 mm mercury for one minute. The test was considered pathological when more than ten petechiae appeared.

Cephalin time was determined according to Waaler (10) The normal range was 63—103 seconds with 95 confidence

Collection of blood Blood was collected from the antecubital vein with sharp silicone treated needles. The first 2—3 ml of blood was discarded and the blood was then allowed to flow freely into lusteroid tubes. In the first tube 1—2 ml was collected, and from this sample the glass capillaries for the whole blood clotting time determinations (see below) were filled In the second tube which contained 1 ml of a 3 trisodium citrate solution 9 ml of blood was collected, immediately mixed and centrifuged at 1 700 g and +4 C. The plasma was transferred to a silicone-treated glass tube by means of a silicone treated pipette and kept in an ice bath until used.

Platelet counting was performed according to Björkman (1)

Thrombin time 0.2 ml of citrated plasma was mixed with 0.2 ml of bovine thrombin (Topostasin) The concentration of thrombin was such as to give a clotting time of 20 seconds with a normal plasma.

Whole blood clotting time A modification of Mayer s method (5) was used. Silicone-treated glass capillaries with an inner diameter of 0.5 mm and 200 mm long were filled with blood sealed at both ends with plasticine, and left in a water bath of 37° C for 4 minutes 5 mm of the capillaries was then broken off every 30 seconds. The time that elapsed before the blood could be drawn out as a thread was regarded as the clotting time. The upper normal limit is 11 minutes.

Dosage of heparinoid

1 The volunteers were given simultaneously single injections of 400 mg intravenously and 1 000 mg intramuscularly

2 For practical purposes, the initial dose given to the patients varied with the time of admission into the hospital. The patients who arrived between 8 a.m. and 3 p.m. received a full dose of 400 mg intravenously and 1 000 mg intramuscularly Those who arrived between 3 p.m. and 10 p.m. received 400 mg intravenously and 750 mg intramuscularly while the patients who were admitted between 10 p. m. and 6 a. m. were given 600 mg intravenously At 8 a.m the second day all patients were given 1 000 mg intramuscularly

750 mg was given intramuscularly to the patients with myocardial infarctions on the third day and the therapy was then discontinued. The patients with venous thrombosis or pulmonary embolism received 1 000 mg intramuscularly on the third and fourth days, and then 750 mg intramuscularly daily until the therapy was discontinued after 7 days.

During the course of the investigation, it became evident that the doses had to be varied in accordance with the body weight. During the last period of the investigation, the initial intramuscular doses were therefore reduced to 875 mg for patients who weighed less than 50 kg and increased to 1.250 mg for patients who weighed more than 75 kg From the third day the doses were reduced with 250 mg

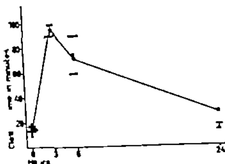


Fig. 1. Whole blood clotting time after simultaneous injection of 400 mg of heparinoid intravenously and 1,000 mg intramuscularly

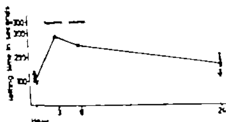


Fig. 2. Cephalin time after simultaneous injection of 400 mg of heparinoid intravenously and 1,000 mg intramuscularly

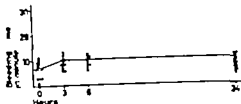


Fig. 3. Bleeding time after simultaneous injection of 400 mg of heparinoid intravenously and 1,000 mg intramuscularly

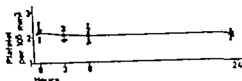


Fig. 4. Platelets after simultaneous injection of 400 mg of heparinoid intravenously and 1,000 mg intramuscularly

Results

Effect on the haemostatic function of single injection

Fig 1 2, 3 and 4 illustrat the effect of single dose of heparinoid on the whole blood clotting time cephalin time, bleeding time, and the number of platelets.

Cephalin time was generally still somewhat prolonged 24 hours after the injections, while the whole blood clotting time in most cases had returned to the initial values 24 hours after the injections. Thrombin time, which is not shown in any figure, exceeded 500 sec. in 6 of the 12 subjects, was more than 100 sec. in 3 and more than 50 sec. in the remaining 3 subjects 24 hours after the injections. Bleeding time was prolonged 3 and 6 hours after the injections in a few cases. The number of platelets did not vary

Effect on the haemostatic function of repeated injections

The effect of repeated injections of heparinoid was studied in the 15 patients who had uncomplicated venous thrombosis. For practical reasons, initial values were obtained only in patients admitted into the hospital during day time. Whole blood clotting time, cephalin time, and thrombin time were then determined every morning before the next injection of heparinoid. Bleeding time, capillary fragility and platelets were determined every second morning Occasionally determinations could not be made because the patient was in the X ray department. As is shown in fig 5 and 6 there were great variations both in the whole blood clotting time and in the cephalin time. Some of these variations probably were caused by the fact that the standard

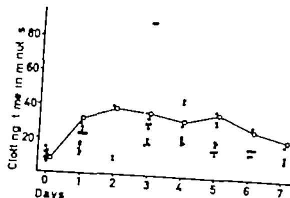


Fig 5 Whole blood clotting time after repeated injections of heparinoid

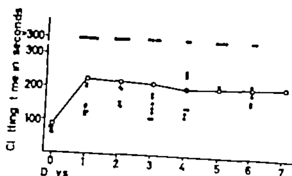


Fig 6 Cephalin time after repeated injections of heparinoid.



Fig 7 Bleeding time after repeated injections of heparinoid.

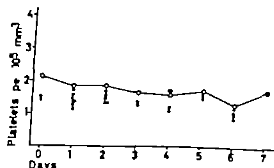


Fig 8. Platelets after repeated injections of heparinoid

Table I Immediate and late therapeutic effect the group of deep venous thrombosis

	Age	Sex	Immediate result	Late result
1	18	♀	+++	+++
2	37	♂	+++	+++
3	40	♂	+++	+++
4	43	♀	+++	++
5	47	♀	+++	+
6	53	♀	+++	+++
7	58	♂	+++	+++
8	65	o	++	++
9	66	♀	+++	++
10	68	♂	+	++
11	70	♀	++	++
12	75	♂	+++	++
13	75	♀	+	+++
14	75	♀	0	died
15	77	♀	0	died

For explanation see text.

dose was given to 10 of the patients irrespective of their body weight. Thrombin time exceeded 200 sec. in about 90 % of the estimations, was less than 100 sec. on 3 occasions, and was never below 50 sec. In some cases there was a slight prolongation of bleeding time (fig 7) The platelets did not vary (fig 8) and increased capillary fragility did not appear during the therapy

Therapeutic effect in the group of "uncomplicated" venous thromboses

The therapeutic effect was evaluated during the period of treatment and by re-examination of the patients 2—3 months after the treatment. The following criteria were used to estimate the immediate and late therapeutic results.

No improvement 0

Disappearance of pain but still daily and nightly oedema +

Oedema only during the day + +

Symptomless + + +

Table II Incidence of loss of hair

	No. of patients	Loss of hair		
		♂	♀	Total
Long-term treatment with heparinoid	18 (11♀ 7♂)	9 (82%)	3 (43%)	12 (67%)
Short-term treatment with heparinoid	51 (29♀ 22♂)	16 (55%)	4 (18%)	20 (40%)
Short-term treatment with heparin	55 (26♀ 29♂)	6 (23%)	5 (17%)	11 (20%)

The result of the treatment was good except in two cases, as will be seen from table I. Of these two patients one was a 75-year-old woman (No 14) She was admitted into the hospital for deep venous thrombosis for the third time in one year Five days after the termination of the heparinoid treatment, signs of arterial occlusion in both legs were observed. A few days later an amputation of the right leg was made. She died in the postoperative period from pulmonary embolism. Patient No. 15 was a 77-year old woman in poor condition. During the treatment she complained of diffuse abdominal distress. Two days after the termination of the heparinoid treatment, sudden abdominal pains occurred which made a laparotomy necessary The patient expired the day after the operation. Autopsy revealed an embolus in the superior mesenteric artery with gangraena in the small intestine There was wide spread arteriosclerosis.

Ten more patients were treated for 7 days. Of these, three had pulmonary embolism, two arterial embolism, four deep phlebothrombosis in connection with malignancy and one superficial thrombophlebitis. The result of the treatment appeared to be positive in 9 of these patients. One patient with an embolus in the axillary artery expired from pulmonary oedema two days after the treatment was terminated. This patient also had an intestinal bleeding which will be discussed later

Side effects

Short-term observations

Of more than four hundred intramuscular injections, ten resulted in haematoma or infiltration in the area of injection. This complication was not serious in any of the cases. Skin eruptions, pains in the joints, or other allergic reactions were not seen. Diarrhoea, which has been reported as a severe toxic reaction (2) was not observed. In one of the patients with gastric bleeding during the therapy however small ulcers in the gastric mucosa were found at autopsy In another patient with intestinal bleeding local hyperaemia of the intestinal mucosa was seen. This will be discussed later

Long-term observation

A revisit was arranged about 3 months after the treatment.

We were able to reexamine 18 of the 25 patients who had been treated for 7 days with heparinoid 50 of the 76 patients

who had been treated for 3 days, and all the 12 normal subjects who had received a single dose

The only important side effect was loss of hair This complication seems to be characteristic of all heparinoid substances and heparin (2 3 4 5 7 9) Loss of hair appeared in these patients 6—12 weeks after the start of the treatment After another 2—4 weeks new hair reappeared A hundred per cent recovery has been reported in most materials In this investigation loss of hair therefore was attributed to the therapy if it started 6—12 weeks after the treatment and if a re-growth appeared in another couple of weeks. The incidence is illustrated in table II In the 12 subjects who received a single injection and in the group of patients treated only with dicumarol, no loss of hair was observed The frequency was higher in the group of patients on long term treatment than in the group on short term treatment with heparinoid There appeared to be a higher frequency in females than in males. The incidence however also seemed to be higher in patients with a good hair growth than in the others. As women usually have more hair and pay more attention to it, the difference between the sexes might not be real The hair reappeared in all patients. Change of colour from grey to black was observed As will be seen from the table, the incidence of loss of hair was less in the 55 heparin treated patients who were questioned than in the hepa rinoid treated patients.

One of the heparinoid treated patients reported loosening of one of his teeth about two months after the treatment As his teeth were in a rather poor condition, it is hard to evaluate the signif icance of this observation. After about two weeks the tooth became firm again, a fact which indicates some relation to the heparinoid treatment. Loosening of teeth has been reported after treatment with mepesulfate (2)

Bleeding during the therapy

Severe bleeding was observed in two patients. In an 86-year-old woman, melaena occurred the day after the termination of the heparinoid treatment. She had received a total of 5.6 g heparinoid during 5 days. The loss of blood was moderate. She died two days later from pulmonary oedema. At autopsy the distal part of the ileum and the proximal part of the colon were found to be filled with clotted blood In this area, hyperaemia of the mucosa, but no ulcerations, was seen. The rest of the intestine and the stomach were normal The other patient was a 69-year-old man admitted into the hospital for myocardial infarction with the clinical picture of prolonged shock. After two days of simultaneous heparinoid treatment and dicumarol administration, he suffered a haematemesis and expired from acute circulatory failure. At autopsy several superficial pinhead sized ulcers were found in the mucosa of the stomach.

In a patient with gastric cancer a subcutaneous haematoma containing approximately 300 ml of blood occurred in the scapular region

In 6 patients an occasional positive benzedine test was observed and microscopic haematuria was seen in 3 patients. Systematic analyses of the stool and urine were not, however performed in all patients on short term therapy

Occasional bleeding during an effective anticoagulant treatment cannot generally be regarded as a toxic reaction In the two patients with gastrointestinal

bleedings discussed above it cannot be excluded, however that the small ulcers in the gastric mucosa and the hyperaemia in the intestinal mucosa were caused by the heparinoid. Shock and advanced age both increase the sensibility to anticoagulants, and those two patients should possibly not have been treated with standard doses of heparinoid.

Field et al. (2) observed diarrhoea in approximately 25 % of the patients who survived a satisfactory observation period. Postmortem examinations of the three patients in their series who expired from this complication revealed superficial ulcerations and hyperemia of the mucosa in the entire intestinal tract. Our patients had no diarrhoea.

Results of the routine laboratory tests

Analyses of the white cells, plasma-creatinine and protein in the urine in the investigated cases revealed nothing which could be related to the heparinoid treatment. Lowered haemoglobin concentration was seen only after bleeding

Discussion

Evaluation of therapeutic effect on thromboembolic diseases is extremely difficult. The localization and extent of the thrombi, the underlying disease, and the tendency to form new thrombi vary Large materials with commensurable control materials would therefore be required to give a satisfactory answer With a drug in our hands such as heparin, which is known to have a beneficial effect, an investigation of this kind is not justified. The present study was therefore limited to the evaluation of the therapeutic effect in a small group of venous thrombosis. In these patients, the new heparinoid seemed to give a rapid release of pain and oedema in the same manner as we have been used to see in heparin treatment. The late results are also comparable with those seen in heparin treatment.

The main side effect of treatment with heparin and heparinoids seemed to be the loss of hair Comparatively little attention has been paid to this toxic reaction. Merz (7) however found an incidence of 50—60 % of loss of hair after heparin treatment. Varying degrees of this complication have been reported after treatment with different heparinoids. Thus Tomascheck (9) observed loss of hair in 100 % and Fischer et al. (3) in 45 % after treatment with "Thrombocid" Hjort and Stormorken (5) in all subjects treated with dextran sulphate Field et al. (2) in 70 of the patients treated with mepesulfate, and Hirschboeck et al. (4) in 19 % after treatment with "Treburon"

There are conflicting opinions as to the relation of the complication to the magnitude of the doses given. Field et al. (2) found that the incidence of loss of hair increased with increasing doses, whereas Hirschboeck et al. (4) found no such correlation. In the present work, there appeared to be a greater incidence of loss of hair in the group on long-term treatment than in the group on short term treatment. The subjects who received a single injection did not show loss of hair

The great discrepancy in the incidence of loss of hair after heparin treatment in Merz's and our materials might be explained by the difference in doses. Our patients received about 25,000 units of heparin daily for 1—3 days, while most of the patients in Merz's material were treated with about 50 000 units a day for

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Surgery During Anticoagulant Treatment

The Risk of Increased Bleeding in Patients on Oral Anticoagulant Treatment

By

Herman Rustad and Erik Myhre

Anticoagulant treatment with dicumarol or indandione preparations is widely used to prevent thrombotic episodes following surgery. Furthermore, an increasing number of patients on long term anticoagulant therapy for coronary disease are admitted for surgical treatment.

The post-operative prophylaxis does not cover the first days after the operation (15) and it would therefore be desirable to operate on patients during anticoagulant prophylaxis.

The aim of the present investigation was to analyze whether this might be safe.

The study was done as a double blind test with random division of patients into equal groups of patients on anticoagulant therapy and control. To further reduce the variables, two standard surgical procedures, cholecystectomy and gastric resection for peptic ulcer were selected for study. Both these procedures were done with the same technic by all surgeons concerned, and as far as possible the surgeons were not told whether or not anticoagulants had been given.

Submitted for publication June 28, 1962.

Material and methods

The material included 20 patients who had cholecystectomy and 40 patients who had gastric resection done.

There was no significant difference in age and sex between the groups.

Cholecystectomy was done during anticoagulant treatment in 7 women with an average age of 56 years and in 3 men with an average age of 55, while 8 women with an average age of 61 years and 2 men with an average age of 55 made up the control group.

Gastric resection was done during anticoagulant treatment in 16 men averaging 48 years of age and 4 women with an average age of 51 years. The control group consisted of 16 men with an average age of 43 years and 4 women with an average age of 41.

Anticoagulant treatment

The treated groups received phenylindandione (Trombantin) before surgery usually starting the day after admission. The anticoagulant effect was determined by the PP (prothrombin-proconvertin) test or in the later part of the series by the TT-test (thrombotest (8)). The patients received an initial dose of 120 mg phenylindandione, and the treatment was carried on under daily control by PP or TT until the level was stable. We aimed for PP or TT concentration of 15—25 % of normal.

6—12 days. It must also be remembered that all of Merz's patients were women in a maternity hospital while the majority of our patients were comparatively old men

For the time being it is not possible to state whether the toxic effect of heparin and heparinoids is limited to the loss of hair The observation of hyperaemia and small ulcers in the gastrointestinal tract might indicate that the reaction is more general Thus question cannot, however be answered until future observations have been made. Until then, it seems reasonable to avoid large doses for prolonged times.

Summary

Heparinoid Geigy 31150 was given to 113 subjects.

It gave a satisfactory anticoagulant effect which after a combined intramuscular and intravenous injection, lasted for 24 hours and which could be maintained by one single intramuscular injection daily

The clinical effect was studied in a small group of patients with crural venous thrombosis and was found to be good

As is the case after treatment with heparin and other heparinoids, loss of hair appeared in the patients about 2 months after the treatment.

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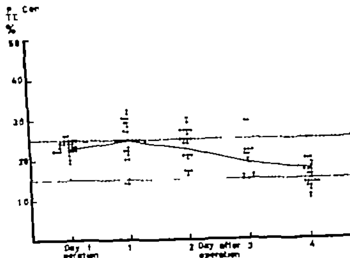


Fig. 1. PP or TT concentration in 30 patients who were given oral anticoagulant treatment preoperatively during and after the operation. The solid line denotes the average values.

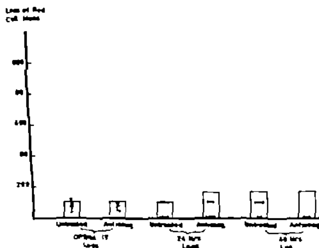


Fig. 2. The operative, external blood loss and the total blood loss during the first 24 and 48 hours after operation, computed as loss of red cell mass, following cholecystectomy. The columns denote average losses.

The total loss during the first 48 hours was 165 ml RCM in both groups, indicating that no significant difference existed.

Fig. 3 shows the blood losses in the gastric resections. *In the untreated group* the average external blood loss was 410 ml whole blood or 175 ml RCM and in the treated group the loss was 400 ml whole blood or 170 ml RCM. There is obviously no significant difference between these values.

The mean loss during the first 24 hours was 330 ml RCM in the group given anticoagulant versus 275 ml RCM in the control group.

The loss during the first 72 hours was 440 *ml* RCM *or about* 1 100 *ml* whole blood in the treated group while the control group had an average loss of 410 ml RCM or about 1 000 ml whole blood.

These differences, although below the error of the method of determination, indicate a slightly increased tendency to

The patients received phenylindandione orally also on the day of operation early in the morning and on the following days. Resorption seemed to be satisfactory even in the early post-operative period

The treatment was continued until the patient was discharged and was then tapered off.

Blood loss measurement

The external blood loss during the operation was determined by a method described elsewhere (10) The blood was extracted from sponges and linen in a washing-machine and the amount of blood in the wash water was determined by photocolorimetry

As Fretheim (4) and others have noted the external blood loss is usually less than the total loss. This is especially the case in gastric surgery where an intestinal loss often takes place In addition a certain loss due to post operative oozing will always occur

These losses were also calculated Blood volume determinations were done before surgery and on the first and the second or third post-operative day The total loss of blood was then computed as the sum of blood volume deficit and the amount of transfused blood

Blood transfusions were generally given to patients who had gastric resection done and occasionally also to patients undergoing cholecystectomy

As the plasma volume is unstable in the post-operative period due to a varying exchange with the extracellular fluid, estimates of the red cell mass (RCM) were used in these calculations.

The RCM determinations were done with ^{51}Cr-tagged erythrocytes according to Sterling & Gray (12) with some minor modifications. For convenience Rh neg type 0 blood, freshly drawn, was used and labeled in vitro with 5 μC ^{51}Cr/ml After labeling the cells were washed twice with normal saline and resuspended in normal saline.

After a blood sample had been withdrawn, 8 ml of the suspension was injected and 10 min. later a second blood sample was drawn from another site. The radioactivity of the blood samples was measured and the RCM computed as discussed in another paper (7)

The standard error of the method in our hands was 2.9 % which means that with a total RCM of 2 000 ml a change in RCM by 170 ml is 95 % significant.

Results

Anticoagulant treatment

The PP or TT values during the operation and post-operative period are shown in fig 1 There was a tendency to increasing values post-operatively because we first followed Storm's (13) suggestion of operating on an increasing level. Later patients were operated on and maintained on a stable level of about 20 per cent.

We noted an increased sensitivity for phenylindandione during the immediate post-operative period when the patients on an average required only about 2/3 of the pre-operative dose. This was more marked after cholecystectomy than after gastric resection.

The patients requirements returned to their pre-operative dosage on the fifth to eighth post-operative day This usually coincided with the return to fairly normal dietary intake.

Blood loss

In patients who had cholecystectomies done the average external blood loss was 225 ml blood or 100 ml RCM in the untreated group and 230 ml blood or 104 ml RCM in the anticoagulated group (fig 2) and consequently no difference was established

The total blood loss during the first 24 hours was 100 ml RCM (225 ml whole blood) in the untreated group and 160 ml RCM (400 ml whole blood) in the group given anticoagulant. This difference however is below the mean error for the method of determining blood volume.

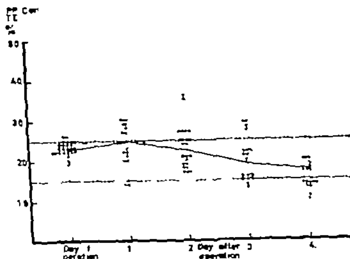


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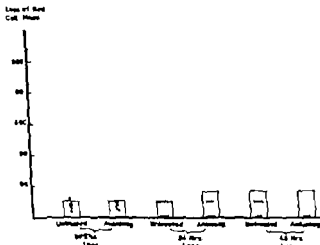


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be avoided by operating under the protection of continuous anticoagulant therapy (5)

The present study shows that surgical treatment during effective anticoagulant prophylaxis is possible without untoward risk of bleeding

It must be mentioned, however that two of the patients on anticoagulant treatment had an operative blood loss of more than 800 ml RCM, corresponding to 2,000 ml of whole blood, and required several transfusions. In one of these patients the TT level at the time of bleeding was 13 per cent. In the other patient the PP level was 24 per cent. His bleeding came immediately after the operation and was probably due to inadequate suture-line hemostasis in the gastroenterostomy A similar gross bleeding also occurred in one patient not receiving anticoagulant treatment.

Careful management and control is required in order to keep the patients at the optimal level of hypocoagulability Daily control of the PP or TT concentration is essential, because overtreatment may easily result in excessive bleeding

The PP (prothrombin-proconvertin) test was used in the first of these patients, but later the TT (thrombotest) method was preferred, because it is more sensitive to the factors IX and X, the excessive depression of which is the most frequent cause of bleeding complications (9)

Summary and conclusion

A group of patients subjected to operation while on anticoagulant therapy has been compared with a control group of patients operated without such treatment, with regard to bleeding during and after the operation. The study entailed two standard surgical procedures, cholecystectomy and gastric resection for peptic ulcer

There was no difference in the operative external blood loss between the two groups.

Blood volume determinations preoperatively and on the second and third post-operative day were done and the total blood loss calculated

No significant difference was found in the total blood loss between treated and untreated patients in the cholecystectomy group. In the gastric resection group a moderately increased tendency to post operative bleeding was noted

The investigation indicates that patients may be safely operated on during anticoagulant therapy at a therapeutic level, as estimated by PP test or TT test, between 15 and 25 per cent.

Reliable laboratory service for daily control of the anticoagulant effect is essential.

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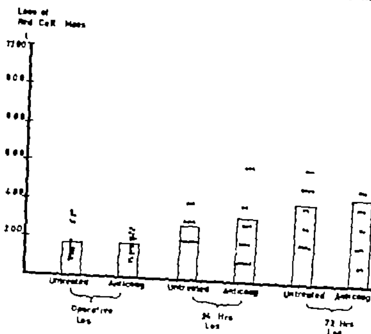


Fig 3. The operative, external blood loss and the total blood loss during the first 24 and 7 hours after surgery computed as loss of red cell mass in patients who had gastric resection done. The columns denote average losses.

post-operative oozing in the patients on anticoagulant therapy

Five patients in the untreated group had losses exceeding 1 000 ml of whole blood while eight patients in the group given anticoagulant lost this amount. One patient in the group given anti coagulant lost more than 2 000 ml of blood and three patients more than 1 500 ml while one patient in the control group lost more than 1 500 ml the first 24 hours.

One patient in the untreated group and two patients in the group given anti coagulant lost more than 2 000 ml whole blood during the first 72 hours

Complications

There were no thrombo-embolic complications and no mortality in either group

Discussion

Several reports indicate that oral anti coagulant therapy with dicumarol or in dandione has an anti thrombotic effect when the PP or TT concentration is in the range of 15 to 25 per cent (1 11)

The hemostatic process as tested by the primary and secondary bleeding time is unimpaired at these levels and consequently no significant hemorrhagic tendency should be present (2)

Therefore surgical intervention should presumably be safe in this range.

The advantages of anticoagulation during surgery are obvious. Although a marked reduction in thrombo-embolic complications is obtained by post-operative anticoagulant prophylaxis (5 6, 14) thromboembolic complications may still occur before the anticoagulant treatment has become effective.

Only by maintaining effective anticoagulation through the operation and the post-operative period is it possible to secure maximal protection against thrombo-embolism

Furthermore, discontinuation of anticoagulant treatment during surgery in patients with arteriosclerotic coronary disease increases the risk of recurrent coronary occlusions. This problem may

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Hemodynamic Response to Exercise During Atrial Flutter and Sinus Rhythm

By

Irma Åstrand T. Edward Cuddy[1] Johan Landegren, Robert O. Malmborg
and Bengt Saltin

The increase in cardiac output in response to muscular exercise in normal men (6) and in dogs (10) has been shown to be largely due to an increase in heart rate. When man is exercising in standing or sitting position, there is an initial increase in stroke volume from the resting level, but little further increase as the severity of the exercise is increased (2, 3 13). In dogs exercised on a treadmill with heart rate controlled artificially the cardiac output is maintained relatively constant although heart rate ranges from 60 to 240 beats per minute, by concomitant alterations in stroke volume (14).

This report concerns the work response of heart rate, cardiac output and oxygen uptake in patient with a paroxysmal atrial flutter. Because the heart rate response to work loads was very different during periods of sinus rhythm and atrial flutter the patient was considered well suited for studies on the influence of variations in heart rate on the cardiac output.

Submitted for publication July 3, 1962.

The patient was a 43-year-old lumberjack (height 173 cm (68 ins) weight 71.5 kg (157 1/2 lbs)) who had had paroxysmal attacks of supraventricular tachycardia diagnosed as atrial flutter for 5 years. The number of attacks and the duration of each attack had gradually increased, and at the time of this study they could appear every one or two days with duration of 1 min. to several hours. At times one attack could continue for many days, maximally a fortnight. During attacks the patient was aware of the rapid heart action, and was troubled with dyspnoea on effort, and after an attack of several hours, he felt very tired. Despite this, he usually was able to carry on with his work. Therapeutic trials with digitalis, quinidine, and pronestyl had been made but did not appreciably affect the number of attacks or the symptoms.

The physical examinations and the laboratory investigations performed at our hospital did not disclose any cardiac disease, except for the arrhythmia. At roentgenological examination, the heart was found to be slightly enlarged, but showed normal configuration. The PBI was 6.1—5.7 µg/ml of plasma, and the metabolic rate at rest was 113 The

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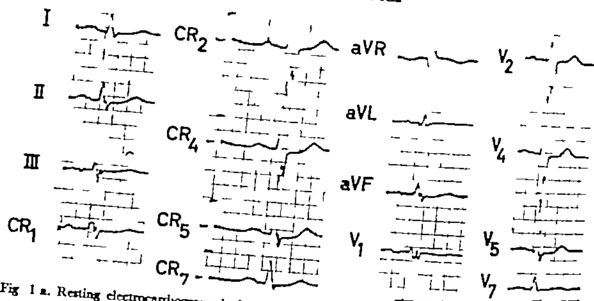


Fig 1 a. Resting electrocardiogram during sinus rhythm. Heart rate 70 min.

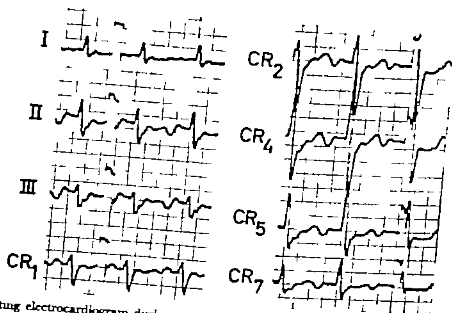


Fig 1 b. Resting electrocardiogram during atrial flutter Atrial rate 300 min. Ventricular rate 150/min.

clinical picture was not consistent with hyperfunction of the thyroid.

The patient was treated with digitalis, but neither this nor different anti-arrhythmic agents significantly influenced the frequency of attacks or the ventricular rate during periods of flutter. At the time of this study the patient was given a maintenance dose of digitoxin, 0.2 mg daily.

The resting ECG during sinus rhythm is shown in fig 1 a. Aside from S—T changes, probably related to digitalis effect, there is no abnormality. Fig 1 b shows the ECG during a period of atrial flutter. The atrial rhythm is regular with a rate of about 300/min. There is a 2 : 1 A—V block and a ventricular rate of about 150/min. At this rate S—T "sagging" and T wave inversion is seen.

Methods

On three occasions during normal sinus rhythm and two occasions during atrial flutter the patient was exercised on a bicycle

Table I Hemodynamic and ventilatory data at rest and during work

Date Sept.	Rhythm	Work load, kpm/min	Atrial rate	Vent. rate	$\dot{V}_{O_2}$, l STPD	$\dot{V}_E$, l BTPS	A-V O_2 diff., ml/min	Cardiac output, l/min	Calculated mean stroke vol., ml	Lactic acid conc., mEq/l
18	Atrial flutter	Rest	312	157	—	—	—	—	—	—
		300	304	153	—	—	—	—	—	—
		600	287	178	—	—	—	—	—	—
		900	283	233	—	—	—	—	—	5.9
20	Sinus rhythm	Rest	—	74	—	—	—	—	—	—
		300	—	102	1.07	23.9	—	—	—	—
		600	—	123	1.62	37.8	—	—	—	
		900	—	154	2.29	52.6	—	—	—	—
25	Sinus rhythm	Rest	—	73	0.53	—	50	7.1	93	—
		300	—	88	1.25	—	111	11.3	128	2.3
		600	—	110	1.67	—	129	12.9	117	2.9
		900	—	142	1.92	—	141	13.6	96	5.1
27	Sinus rhythm	Rest	—	75	0.44	16.5	75	5.8	77	—
		300	—	90	1.00	24.5	97	10.3	114	—
		600	—	121	1.42	38.0	117	13.0	107	—
		900	—	154	1.83	61.2	134	13.7	89	—
28	Atrial flutter	Rest	322	161	0.41	13.53	—	—	—	—
		300	307	162	1.14	27.04	111	10.3	63	2.5
		600	300	182	1.57	42.48	126	12.5	69	3.0
		900	291	207	2.11	74.07	—	—	—	—
		900	283	222	2.26	77.45	167	13.5	61	7.0

ergometer for about 7 min. at each of 3 work loads: 300, 600 and 900 kpm/min. Rest periods of at least 10 min. were given between each exercise load. The patient had no particular symptoms except mild dyspnoea at the heaviest load. On two occasions during sinus rhythm and once during the arrhythmia, A—V O difference and cardiac output were calculated employing the acetylene technique (7) previously used (4) and validated during exercise (1). Arterial pressure was recorded with an Elema strain gauge on Mingograph recorder from teflon catheter introduced percutaneously in the left brachial artery on one work test during flutter. Ventilation was measured and oxygen consumption was calculated using the open-circuit technique. Expired air was collected in Douglas bags during the last 1 or 2 min. of each exercise period and it was analyzed for O and CO content with the Haldane apparatus. Lactic acid estimations on venous blood were performed with modification of the Barker and Summerson technique (11). Precordial ECG leads were continuously recorded during each experiment.

Results

The results are summarized in table I. The *atrial and ventricular rates* given are averages of the values measured during 2 min. at rest and during the last 2 min. of each exercise load. At rest during attacks of atrial flutter the atrial rate was 312—322 beats/min. with 2 : 1 A—V conduction block and a ventricular rate of 157 to 161. During work with in-

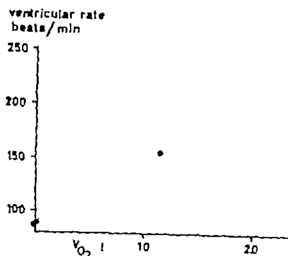


Fig. 2. Ventricular rate in relation to oxygen uptake ($\dot{V}_{O_2}$) during sinus rhythm and atrial flutter
● = sinus rhythm.
○ = flutter

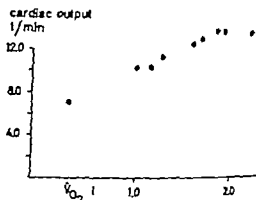


Fig. 3. Cardiac output in relation to oxygen uptake ($\dot{V}_{O_2}$) during sinus rhythm and atrial flutter.
● = sinus rhythm.
○ = flutter

creasing load the atrial rate declined successively to about 280/min. at the heaviest work load. During all grades of work intermediate conduction delay of the Wenckebach type was seen, producing an irregular ventricular action. On the heaviest work load the A—V conduction was at times 1 : 1 thus resulting in a ventricular rate of about 260/min. On three occasions short series of markedly altered QRS-complexes were seen. It cannot be definitely established if this was due to aberration of supraventricular beats or to ventricular tachycardia.

The response of ventricular rate to work load during sinus rhythm and during flutter can be seen in fig. 2. At both conditions the ventricular rate increased linearly with oxygen uptake. The mean ventricular rate during flutter exceeded the rate during sinus rhythm by approximately 84 beats/min. at rest and by 53—79 beats/min. during different work loads.

The *oxygen consumption* was approximately the same at each level of work during both rhythms as shown in fig. 2. Fig. 3 shows that the relation between *cardiac output* and oxygen consumption at different levels of work was approximately the same during flutter and sinus rhythm. The same cardiac output could be maintained at each work load during sinus rhythm as well as during atrial flutter despite the very fast ventricular rate during flutter. Calculated mean stroke volume was lower during the period of arrhythmia and remained approximately constant at different work loads. The cardiac output did not increase as much with increasing work load from 600 to 900 kpm/min. as from 300 to 600 kpm/min. This might be a sign that the patient worked not far from his maximum. The decreasing mean stroke volume with increasing work load during sinus rhythm may be another sign of that (cf. the concentration of lactic acid).

The *arterial pressure* showed considerable variation in systolic and diastolic values during work when the ventricular

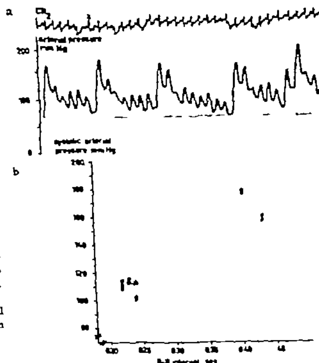


Fig. 4. a. Electrocardiogram and arterial pressure during atrial flutter when cycling on a work load of 900 kpm/min. The crosses ( ) show two corresponding points on the curves. b. The relationship of R—R interval to subsequent systolic arterial pressure. The values have been measured on the basis of fig. a.

rate became more rapid and irregular (fig. 4). Fig. 4 b shows that the variation in blood pressure was related to beat to beat variation in ventricular rate. When the preceding R—R interval was less than 0.25—0.27 sec. the subsequent systolic and pulse pressure was much reduced. These R—R intervals correspond to ventricular rates of 222—240/min. During periods of 1 1 conduction, which seldom exceeded 2—3 sec. in duration, the blood pressure fell continuously. During these periods the R—R intervals were 0.23 sec. and the ventricular rate was 260/min.

Discussion

Acceleration of the ventricular rate due to increased A—V conduction is a well recognized consequence of exercise in cases with atrial flutter and the danger of producing 1 1 conduction during exercise has been emphasized (11). In this case atrial rate was moderately slowed during exertion.

Harvey et al. (9) studied two patients with atrial flutter and without congestive heart failure. Both had diagnosed cardiac disease and were older than our subject. Cardiac output response to very mild exercise was the same during flutter and sinus rhythm but the ventricular rate was in each case below 100/min. In contrast to this Cramér et al. (5) among others found a lower cardiac output during exercise with auricular fibrillation than with sinus rhythm in 8 patients with mitral stenosis.

The varying arterial systolic and pulse pressure observed during exercise when our subject had atrial flutter was related to changes in the R—R interval and therefore in the time available for diastolic filling. The rough correlation ob-

served between pulse pressure and stroke volume (8) permits the beat to beat changes in blood pressure to be related to parallel changes in stroke volume. In this subject a critical ventricular rate might be defined above which stroke volume was sharply reduced. Only during rapid ventricular rate resulting from 1:1 conduction did the arterial pressure indicate progressive failure of stroke volume. It is indeed remarkable that this man's heart could produce sufficient stroke volume to maintain the increased flow demanded by exercise at a ventricular rate averaging 220/min.

Since the increase in oxygen consumption during exercise was similar during sinus rhythm and flutter, it must be inferred that the mechanical efficiency was at the same level. No direct measurements of oxygen debt were made, but venous lactic acid concentrations were not statistically different at the three work loads during flutter compared to those during sinus rhythm (table I).

The cardiac output was practically identical at comparable levels of work. These findings suggest that the peripheral oxygen requirements serve as the major determinant of blood flow. They also suggest that the cardiac output is to a very large extent regulated according to the demands of the tissues and is in this case relatively independent of heart rate. By an accurate adaption between stroke volume and heart rate the cardiac output might remain unchanged when, as a consequence of a fast atrial arrhythmia, the ventricular rate response to physical activity is greatly altered.

Conclusion and summary

The exercise response of oxygen consumption, cardiac output and cardiac rate has been measured in a 43-year-old man during normal sinus rhythm and during paroxysmal atrial flutter. Ventricular rate was fast at rest during atrial flutter and increased up to 260 during the heaviest work compared to 154 during sinus rhythm. Despite the rapid ventricular rate, cardiac output and oxygen consumption were not measureably different from the values during sinus rhythm. The suggestion is made that the peripheral oxygen requirements of exercise were unchanged during the arrhythmia and served as the major determinant of blood flow.

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The Seventeenth Annual Symposium on Fundamental Cancer Research February 20 21 and 22 1963

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On June 3—5 1963 a Congress with international participation on the subject of Vitamin Research in Nutrition will be held in Prague

Programme

1st day Introduction on the importance of vitamins in nutrition Protection of vitamins in technological processes and during the preparation of foods.

2nd day A. Main shortcomings of vitamin supplies and their sequelae on health B Scientific bases for the use of vitamins in the prevention and treatment of nutritional disorders and some mechanism of this action.

3rd day Problems of the biochemical estimation of vitamins in foodstuffs and bio chemical material (panel discussion)

All communications regarding the congress should be addressed to Miroslav Bohdal M.D Institute of Human Nutrition Praha Krč, Budějovická 800

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and the Department of Medicine IX (Head K. Aas, M. D.)
Ullevaal Hospital, Oslo, Norway

The 17 Hydroxycorticosteroid Response to Corticotrophin, Metopiron and Bacterial Pyrogen

By

Truls Brinck Johnsen, Jan H. Solem, Kari Brinck Johnsen and Per Ingvaldsen

Although numerous experiments have been designed to investigate the mechanisms concerned with the release of ACTH from the pituitary the various factors involved have not been clarified. We know that a fall in steroid level will increase ACTH output, and an elevation of this level will inhibit it. Such a simple mechanism is, however no longer accepted as the only factor concerned in the response to stress (6) N acute drop in blood corticoids has been demonstrated during stress, and most probably other factors are responsible for the changes in ACTH secretion following a stress stimulus.

I assessing the capacity of the pituitary to store and release corticotrophin some direct measurement of the circulating hormone would be ideal. The bioassay techniques at present vailable remain, however principally research tools. An indirect estimation of pituitary adrenocorticotrophin reserve can be btained by studying the pituitary response to lowered levels of circulating cortisol induced by means of an inhibitor of 11β-hydroxylation SU-4885 or metopiron (1) In normal subjects, the increased secretion of pituitary ACTH in response to the lowered level of plasma cortisol is manifested by an enhanced excretion of urinary 17 hydroxycorticosteroids (13) Quantitative measurements of the pituitary response to various stressful stimuli and the development of a standardized test to measure this response have been the basis of several studies. An increase in the release of ACTH and in the concentration of plasma 17-hydroxy corticosteroids or urinary 17-ketogenic steroids following the administration of various pyrogens has been demonstrated (4 5 14 19 24)

The present study was designed to further evaluate the usefulness of changes in 17-hydroxycorticosteroids following metopiron administered orally or intravenously and bacterial pyrogen administered intravenously as aids in clinical

Submitted for publication July 3, 1962.

Table I The normal response to the oral metopiron test (10 subjects)

Subject no.	Urinary excretion of total 17-OHCS (mg/24 hrs)		
	Day − 1	Metopiron	Day + 1
1 A	3 0	16.4	22.3
2 A	4.0	18.6	23 4
3 A	4 6	10.3	23 1
4 A	2.9	17 1	28.9
5 A	5 4	11 1	29.5
6 A	5.2	18.2	28.3
7 A	4.3	14.8	25 1
8 A	5.7	19 0	29.2
9 A	1.8	17 7	22.3
10 A	6.7	14 1	22.4

Table II The normal response to the intravenous metopiron test (10 subjects)

Subject no.	Urinary excretion of total 17-OHCS (mg/12 hrs)			
	Day 1 (control values)		Day 2 (metopiron given)	
	1st 12 hrs	2nd 12 hrs	1 t 12 hrs	2nd 12 hrs
1 B	3.2	1 7	6.3	5.5
2 B	4.3	1.5	7.2	4 1
3 B	2.8	2.3	7.3	6 1
4 B	2.5	2.6	9 6	5 1
5 B	1 4	0.9	4.6	1.6
6 B	1.8	1 6	4.2	2.7
7 B	2 1	1 1	4 7	2 1
8 B	1 7	2.4	3 7	3.3
9 B	4.2	2 1	7.3	2.9
10 B	1 4	0.7	5.3	4 0

diagnosis and as an indicator of pituitary adrenal activation in control subjects and in the evaluation of patients with various endocrine disorders Intact adrenals are necessary for the measuring of the pituitary response to metopiron and pyrogen accordingly in all patients the adreno-cortical response to ACTH was compared with the results of "the metopiron test and the pyrogen test"

Methods

Metopiron test

The oral metopiron test was conducted over a period of three days. On the first day a baseline 24-hour urinary excretion of 17 hydroxycorticosteroids (17-OHCS) was determined. On the second day the drug was administered orally in a dosage of 750 mg every four hour altogether 6 doses. The 24-hour urinary excretion on the second and the third day was then compared with the values determined on the first day The results of the test in ten patients with non-endocrine disorders is shown in table I

The intravenous metopiron test was conducted over a period of two days. Two g of metopiron-ditatrate dissolved in 500 ml of 5 glucose solution was given intravenously for 4 hours beginning between 7 and 8 a. m. Urine was collected for two consecutive 12 hour periods beginning at 7 a. m. Specimens were collected for similar periods on the previous day Urinary 17-OHCS were measured in these four 12-hour urinary collections, and the values compared Table II shows the results of the test in ten patients with non-endocrine disorders.

ACTH test

The four-hour intramuscular ACTH test was performed according to the technique of Solem et al. (20) Forty units of a repository ACTH preparation (Jaton prolongatum) was given intramuscularly at 8 a. m. The adrenal response was estimated by measuring the plasma 17-OHCS-concentration just before the injection and four hours later The maximum value for 17-OHCS attained in this standard ACTH test in a control group of 62 subjects was found to vary from 20 4 to 40.2 μg/100 ml plasma.

Chemical methods

The level of plasma and total urinary 17 hydroxycorticosteroids (17-OHCS) was determined by the Eik Nes modification (5) of the methods described by Nelson and Samuels (15) and Glenn and Nelson (7) using the Porter and Silber (17) color reaction.

Pyrogen test

The pyrogen test was performed by injecting 0.50 µg of a bacterial pyrogen (Organon) intravenously at 8 a. m. and drawing blood samples immediately before, and 4 hours after the injection. During this time the temperature was measured at intervals. The free plasma 17-OHCS were determined in the two samples. Fig. 1 shows the result of the test in 60 patients without any endocrine disorder or disease known to interfere with the metabolism of glucocorticosteroids. None had been running an increased temperature in the days previous to the pyrogen test. In the majority of the subjects the administration of the pyrogen was followed in one to tw hours by an elevation of temperature to 38° C and higher which persisted for three to four hours

During the test, but not necessarily followed by pyrexia, chills were observed and the patient complained of muscle pains and headaches nausea and vomiting were rarely observed. These last symptoms have been observed mainly in elderly fragile women, some of whom had been rather distressed during the test. Hypotension did not develop, and it was never necessary to terminate the test by administrating water-soluble preparation of hydrocortisone intravenously It might hav been an advantage to standardize the test with dose of 0.005 µg per kg body weight according to the technique of Ferriman and Page (5) instead of modifying it by giving standard dose of 0.50 µg to all patients.

There appeared to be proportional relationship between the febrile response and the increase in plasma 17-OHCS levels. Fig. 1 shows the relationship between the temperature and 17-OHCS in plasma t 12 noon The straight line fitted to the data by means of the least squares method has the equation

$$Y = 7.749X - 275.17$$

A graphical analysis of the relationship between the plasma 17-OHCS t 8 a. m. and at 12 noon, showed no indication of any correlation. Accordingly there is no reason for correlating the difference between the tw values, rather than the 12 noon alue with temperature Nevertheless, all the subjects

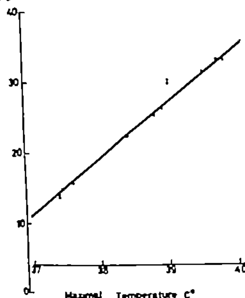


Fig. 1 Plasma 17-OHCS 4 hours after pyrogen injection.

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Results

The pituitary function tests ha been performed in twenty-five patients. Table III shows the response to the tests compared with the basal levels of plasma 17-OHCS and the response to the standard ACTH test. I most cases only the oral or the intravenous metopiron test was carried out. In some of the cases where both tests were performed, one of them is not reported since it unfortunately was spoiled when urine collection was unsuccessful. The pyrogen test was performed in all patients. Case O represents non-endocrine disorder where all tests were

Table I The normal response to the oral metopiron test (10 subjects)

Subject no.	Urinary excretion of total 17-OHCS (mg/24 hrs)		
	Day − 1	Metopiron	Day + 1
1 A	3.0	16.4	22.3
2 A	4 0	18.6	23 4
3 A	4 6	10.3	23 1
4 A	2 9	17 1	28.9
5 A	5 4	11 1	29.5
6 A	5.2	18.2	28.3
7 A	4.3	14.8	25.1
8 A	5 7	19 0	29.2
9 A	1.8	17 7	22.3
10 A	6.7	14 1	22.4

Table II The normal response to the intravenous metopiron test (10 subjects)

Subject no.	Urinary excretion of total 17-OHCS (mg/12 hrs)			
	Day 1 (control values)		Day 2 (metopiron given)	
	1st 12 hrs	2nd 12 hrs	1st 12 hrs	2nd 12 hrs
1 B	3.2	1 7	6.3	5.5
2 B	4.3	1.5	7.2	4 1
3 B	2.8	.3	7.3	6 1
4 B	2.5	2.6	9 6	5 1
5 B	1 4	0 9	4 6	1 6
6 B	1.8	1 6	4.2	2.7
7 B	2.1	1 1	4 7	2 1
8 B	1 7	2 4	3 7	3.3
9 B	4.2	2.1	7.3	2.9
10 B	1 4	0.7	5.3	4.0

diagnosis and as an indicator of pituitary adrenal activation in control subjects and in the evaluation of patients with various endocrine disorders. Intact adrenals are necessary for the measuring of the pituitary response to metopiron and pyrogen, accordingly in all patients the adreno-cortical response to ACTH was compared with the results of "the metopiron test and the "pyrogen test"

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Chemical methods

The level of plasma and total urinary 17 hydroxycorticosteroids (17 OHCS) was determined by the Eik Nes modification (3) of the methods described by Nelson and Samuels (15) and Glenn and Nelson (7) using the Porter and Silber (17) color reaction.

Pyrogen test

The pyrogen test was performed by injecting 0.30 μg of a bacterial pyrogen (Organon) intravenously at 8 a. m. and drawing blood samples immediately before and 4 hours after the injection. During this time the temperature was measured at intervals. The free plasma 17-OHCS were determined in the two samples. Fig. 1 shows the result of the test in 60 patients without any endocrine disorder or disease known to interfere with the metabolism of glucocorticosteroids. None had been running an increased temperature in the days previous to the pyrogen test. In the majority of the subjects the administration of the pyrogen was followed in one to two hours by an elevation of temperature to 38° C and higher which persisted for three to four hours.

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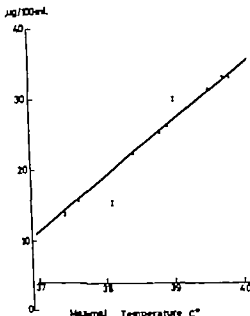


Fig. 1. Plasma 17-OHCS 4 hours after pyrogen injection.

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Results

The pituitary function tests have been performed in twenty-five patients. Table III shows the response to the tests compared with the basal levels of plasma 17-OHCS and the response to the standard ACTH test. In most cases only the oral or the intravenous metopyrone test was carried out. In some of the cases where both tests were performed, one of them is not reported since it unfortunately was spoiled when a urine collection was unsuccessful. The pyrogen test was performed in all patients. Case O represents a non-endocrine disorder where all tests were

Table III The results of the metopiron tests the pyrogen test and the ACTH test in 25 patients with endocrine disorders

Diagnosis	Case no.	Sex	Age (yrs)	Comments	Control values Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)	Metopiron test (intravenous) Urinary 17 OHCS Day 1 and 2: 1st and 2nd (mg/12 hrs)	Metopiron test (oral) Urinary 17-OHCS Day 1 2 and 3 (mg/24 hrs)	Pyrogen test Plasma 17 OHCS 8 and 12 a.m. (μg/100 ml)	ACTH test Plasma 17 OHCS 8 and 12 a.m. (μg/100 ml)
Neurosis	0	♀	66	Control	12.5 11.5	3.2 1 7 6.3 5.5	5 0 16.4 22.5	13.3 29.0	15 0 38 4
Panhypo-pituitarism	1 A	♀	45	Mammary carcinoma with metastasis. Preoperative tests	12.0 6 6	—	—	9.0 17.5	4.2 19.2
	1 B			The same tests performed 5 wks after hypophysectomy	—	—	—	2.9 3.4	4.1 6.4
	2 A	♀	50	Acromegalia. Preoperative tests	8.7 6 4	—	—	11.8 26.1	10.1 33.1
	B			The same tests performed 6 wks after hypophysectomy	—	—	—	3.7 3.4	0.9 2.3
	3	♀	38	Cranio-pharyngioma	9.1 8 1	—	4.9 5.2 3.2	12 1 19.9	8.5 24.5
Partial hypo-pituitarism	4	♀	52	Etiology obscure	8 0 6.9	—	5.7 5.2 2.9	10.7 18.8	8.8 22.2
	5	♂	60	Etiology obscure	8.6 7 7	—	2.1 3 7 1 0	7.0 19 1	6.4 28.8
	6	♀	42	Cranio-pharyngioma	15.3 9.2	2.2 1.5 1.5 1.5	2.7 4.7 10.7	12.3 30.8	10.4 37.0

Table III (cont.)

Diagnosis	Case no.	Sex	Age (yrs)	Comments	Control values Plasma 17-OHCS 8 and 12 a.m. (µg/100 ml)	*Metopiron* test (intravenous) Urinary 17-OHCS Day 1 and 2 1st and 2nd (mg/12 hrs)	*Metopiron* test (oral) Urinary 17 OHCS Day 1, 2, and 3 (mg/24 hrs)	Pyrogen test Plasma 17-OHCS 8 and 12 a.m. (µg/100 ml)	ACTH test Plasma 17-OHCS 8 and 12 a.m. (µg/100 ml)
Partial hypopituitarism	7	♂	45	Craniopharyngioma	15.4 13.4	1.3 1.4 1.3 6.0	—	12.1 23.0	10.4 40.0
	8 A	♂	55	Acromegalia (before treatment)	16.3 16.4	4.8 3.6 7.5 4.6	—	7.0 19.0	16.5 22.7
	8 B			4 wks after the pituitary irradiation (3,000 [illegible] 4 wks)	—	4.7 2.9 3.6 4.6	5.6 14.6 13.5	6.8 18.8	20.3 34.9
	9	♀	63	Chromophobe adenoma of the pituitary gland	13.4 8.8	1.4 1.8 2.4 1.2	3.2 4.9 9.1	12.5 7.3	8.3 50.0
Diseases of the cerebral nervous system	*10*	♂	*19*	*Contusio cerebri*	*18.2* 17.7	4.1 2.1 3.8 13.1	3.6 3.6 3.4	23.2 30.3	*17.0* 38.8
	11		32	Encephalitis (sequelae)	4.6 1.0	—	3.0 7.9 8.9	4.3 9.4	8.6 23.8
Diseases requiring treatment with tranquilizers	12	♂	30	Psychopathia. Treatment with nozinan 50 mg t.i.d. for 6 mos	10.1 8.2	5.0 1.9 2.7 0.9	6.0 6.1 2.5	8.5 19.2	12.9 23.1
	13		23	Psychopathia. Treatment with nozinan 50 mg t.i.d. for 12 mos	19.0 18.3	1.9 0.8 0.7 3.0	5.4 6.1 5.3	15.8 27.3	21.0 33.2

Table III (cont.)

Diagnosis	Case no.	Sex	Age (yrs)	Comments	Control values Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)	Metopiron test (intravenous) Urinary 17-OHCS Day 1 and 2 1st and 2nd (mg/12 hrs)	Metopiron test (oral) Urinary 17-OHCS Day 1 2 and 3 (mg/24 hrs)	Pyrogen test Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)	ACTH test Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)
Diseases requiring treatment with tranquilizers	14	o	38	Neurosis. Treatment with meprobamat 0.40 mg t.i.d. for 4 wks	12.1 8.9	3.0 1.6 2.9 2.0	—	10.0 22.2	11 1 26.0
Primary hypothyrosis	15	♂	70	An advanced case of primary hypothyroidism	15.3 8.5	—	1.0 3.4 9 0	14 1 .3.8	6.9 29.0
	16	♀	66	A case of primary hypothyroidism of moderate degree	10.9 5..	—	2.7 6.2 24.9	9.0 26.4	5.7 29.8
Hyper thyrosis	17	♂	65	An untreated case of thyrotoxicosis	13.8 11 1	4.0 3.0 4.5 2.6	4.8 4 1 15.0	16.8 14.8	17.1 28.7
Diseases requiring treatment with glucocorticosteroids or ACTH	18 A	♀	66	Temporal arteritis before steroid treatment	7 9 7 1	—	—	6.8 22.0	5.1 29.9
	18 B			After 10 day treatment with prednisone 15 mg q.i.d.	—	—	—	9.0 12.0	6 9 27.0
	19	♂	64	Carcinoma of the prostatic gland. After treatment with cortisone 50 mg daily for 14 mos	12.9 11 1	1.8 0 4 1.3 1.3	—	19.4 2 4	15.1 28.4
	20	♀	59	Rheumatoid arthritis. After treatment with prednisone 10 mg daily for 20 mos	4 1 3 7	—	3 7 6.3 10.3	5.5 19 1	4.1 22.4

Table III. (cont.)

Diagnosis	Case no.	Sex	Age (yrs)	Comments	Control values Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)	Metopirone test (intravenous) Urinary 17-OHCS Day 1 and 2 1st and 2nd (mg/12 hrs)	Metopirone test (oral) Urinary 17-OHCS Day 1 2 and 3 (mg/24 hrs)	Pyrogen test Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)	ACTH test Plasma 17-OHCS 8 and 12 a.m. (μg/100 ml)
Diseases requiring treatment with glucocorticosteroids or ACTH	21	♂	59	Bronchial asthma. After treatment with prednisone 12.5 mg daily for 5 wks	6.3 5.9	—	1.8 1.2 4.2	11.2 17.0	6.7 26.4
	22	♂	60	Sarcoidosis (Boeck). After treatment with prednisone 10 mg daily for nearly 2 yrs	13.6 9.5	—	7.4 6.8 6.2	8.0 14.8	10.8 23.1
	23	♀	48	Bronchial asthma. After treatment with prednisone 10 mg daily for nearly 5 yrs	7.0 6.1	—	2.1 3.6 8.1	7.4 18.4	10.6 21.8
	24 A	♂	58	Acute gouty arthritis before hormone therapy	13.9 8.0	—	—	13.6 33.6	8.4 37.4
	24 B			3 days after 6 days therapy with repository ACTH "prolongatum" 20 IU b.i.d.	11.8 7.7	—	—	13.0 13.9	—
	24 C			10 days after the ACTH therapy outlined above	—	—	—	9.0 14.2	—
Cushing syndrome	25	♀	59	The diagnosis of bilateral adrenal hyperplasia later confirmed by operation	15.9 13.6	— —	14.7 29.0 43.1	16.2 27.5	30.3 65.5

normal, and the responses to the tests in the other patients can be compared with those in this control subject to facilitate the reading of table III

Discussion

The results of the present studies demonstrated the diagnostic and investigative usefulness of the metopiron test and the pyrogen test in disorders of the pituitary gland These tests seem to be particularly useful as diagnostic aids in cases where hypopituitarism is suspected and where the demonstration of a decreased corticotrophin reserve is aimed at. In such cases there appears to be no place for the potentially dangerous insulin tolerance or water loading tests (2) and an ACTH test may even be misleading (11)

The metopiron test and the pyrogen test are of no value as pituitary function tests in cases of adrenal atrophy and unresponsiveness to ACTH We have included two hypophysectomized cases in our series to demonstrate this fact no conclusions can be drawn from the lack of response to the pyrogen test in those cases where no response to the standard ACTH test was obtained (table III case 1 B and 2 B)

Case 3 4 and 5 were patients with partial hypopituitarism who presented symptoms and signs of failure of gonadotrophins and thyrotrophin only The standard ACTH test was normal There was a very low response to an oral metopiron test in all three patients indicating an impaired ACTH releasing mechanism. The response to the pyrogen test was normal It is conceivable that inhibition of cortisol secretion by metopiron might provide a relatively mild stimulus to ACTH secretion and that a stress like an injection of a pyrogen might provide a more intense stimulus. It is possible, however that this finding reflects the view that the normal pituitary response to a stress does not involve those centers postulated to control the reciprocal pituitary-adrenal interrelationship (10) This last concept is supported by the observation of van Wyk et al (23) that certain patients with pituitary stalk sections failed to respond to metopiron, but retained their ability to react to pyrogen Most probably patients reacting in this way do have a "limited pituitary reserve" but do not need large amounts of steroids prophylactically in the face of stress. Cases 6 7 and 8 who presented no clinical signs of pituitary insufficiency were supposed to belong to the same category Case 9 presented no clinical signs of hypopituitarism, but in this patient the pyrogen test as well as the metopiron test showed no response.

Six weeks after a brain injury the pituitary function tests were performed in case 10 The response to the pyrogen test was normal. There was no response to the oral metopiron test, the intravenous test showed a definite response, but normal levels of urinary 17-OHCS were reached at the end of the test. The oral test therefore seems to be more sensitive than the intravenous application of metopiron for the detection of defects in ACTH secreting efficiency Case 11 had had an acute primary non-bacterial encephalitis and was only moderately inconvenienced on follow-up 10 months later when the test was carried out. There were no symptoms nor signs of hypopituitarism. The tests, however revealed a defect in the ACTH releasing mechanism

Cases 12 and 13 had been under a long term treatment with nozinan a pheni-

thiazine derivative, when the tests were performed. The oral metopiron test showed no response, the pyrogen test was normal, a combination encountered in the partial hypopituitarism diagnosed in cases 3 to 5 and also observed in cases 6 to 8 and discussed in this connection. In case 14 meprobamate is shown to produce the same phenomena. The ability of nosman and meprobamate to block the increase of urinary 17-OHCS normally caused by metopiron, points towards an interrelationship between the brain centers affected by psychotropic drugs and those concerned with the release of corticotrophin in response to cortisol deprivation. A similar effect has previously been reported after administration of chlorpromazine (8, 21)

In case 13 the response to the intravenous metopiron test was normal, a result similar to that obtained in case 10 where the patient also failed to respond to the oral test. These findings may reflect a slightly greater sensitivity of the oral metopiron test as compared with the intravenous test in differentiating levels of pituitary ACTH reserve, as previously suggested by Hamwi et al. (9) It might, however be a question of the doses given.

I case 15 the poor response to the metopiron test is consistent with a hypofunction of the pituitary gland secondary to the hypometabolism in advanced primary myxedema, as we have already suggested elsewhere when discussing this case in more detail (21) The result of the test might, however also be explained by the slowed hepatic clearance of cortisol from the circulation known to occur in hypothyroidism (16) In case 16 where the symptoms were moderate nd of relatively short duration, the response to the metopiron test . delayed, but normal levels of urinary 17 OHCS were observed on the third day

Case 17 was an untreated thyrotoxic patient in whom the responses to the metopiron tests and the pyrogen test were negligible Theoretically a rapid removal of cortisol by the liver in hyperthyroidism might result in a depletion of the p tuitary corticotrophin reserve (8) We want to stress, however that the interpretation of the findings in both myxedema and hyperthyroidism following the metopiron and pyrogen test is difficult, and any simple explanation for these complex phenomena at this time would un doubtedly represent an oversimplification.

Cases 18 to 23 represent steroid-treated patients. W th the exception of case 18 they all showed a lack of response or a very poor response to metopiron but responded normally to a pyrogen test. In these cases it is possible that a stressful procedure such as a surgical operation could provoke the release of an appropriate quantity of ACTH from a pituitary gland which is unresponsive to the stimulus provided by diminishing circulating levels of cortisol. Patients can present limited pituitary reserve" as judged by the result of the metopiron test, but nevertheless be able to survive surgical procedures without receiving supportive steroid therapy (13) — an observation which we have been able to confirm (21) It therefore seems that the value of th metopiron test (as standard ized by Liddle et al. (13)) as a preoperative screening test in steroid treated patients is limited in that a normal result of the test indicates an intact ACTH eleasing mechanism.

Table IV shows the response to the pyrogen test preoperatively n ten patients

Table IV The plasma 17-OHCS response to the pyrogen test and to surgery (10 patients)

Case no.	Pyrogen test. Plasma 17 OHCS µg/100 ml 8 a.m. 12 a.m.	The surgical intervention	Plasma 17-OHCS µg/100 ml on the day of operation. Before initiating anesthesia and at the end of surgery
1 C	13.6 38.3	Pneumonectomy	23 4 34.8
2 C	13.6 33 6	Mammary amputation	8.4 37 4
3 C	12 1 31.2	Arthroplasty of the hip joint	16 4 33.9
4 C	11.6 17.8	Pneumonectomy	9.5 25.9
5 C	11 6 25.1	Cholecystectomy	17 9 25.5
6 C	15.3 30.0	Hemicolectomy	20.2 37 7
7 C	14.9 31.2	Cholecystectomy	11.2 32.2
8 C	11.8 31 1	Pyelolithotomy	9 6 29 0
9 C	7.5 18.9	Pyel lithotomy	16 1 34.3
10 C	9.5 20.5	Pyelolithotomy	13 6 23 6

17 OHCS levels during the operation The blood samples were drawn just before the anesthesia was initiated and then at the end of the surgery We are acquainted with the fact that the maximal plasma 17-OHCS concentrations usually are found some hours after the end of the operation (22) but have chosen the routine outlined above for practical reasons Most probably a pyrogen test would be more appropriate to evaluate which of the steroid treated patients should be covered prophylactically with additional corticoids when operated upon. How reliable, however this last test might be in predicting the pituitary response to a surgical procedure remains to be seen.

Case 18 is a steroid-treated patient where a standard ACTH test is normal — and in case of operation would have given misleading information. This patient had been treated with relatively heavy doses of prednisone, whereupon the pituitary test was performed and revealed a defective endogenous capacity for corticotrophin secretion. The response to the pyrogen test was very poor This patient we think should have been covered with additional corticoid therapy in the event of an operative procedure.

In case 24 there was no response to the pyrogen test after six days of treatment with ACTH This finding reflects the experience that prolonged administration of corticotrophin may also inhibit the release of endogenous corticotrophin and interfere with the normal responsiveness of the pituitary-adrenal system to stress (11)

Case 25 presented an untreated Cushing's syndrome when the pituitary tests were carried out The diagnosis of bilateral adrenal hyperplasia was later confirmed at operation. The basal plasma 17 OHCS were elevated. The response to the standard ACTH test was much higher than observed in patients with non-endocrine disorders (20) The result of the oral metopiron test was characteristic for adreno-cortical hyperplasia an elevated 17-OHCS output which is further increased by metopiron administration. The response to metopiron may prove useful as a clinical test for differentiating

adrenal hyperplasia from carcinoma, since the neoplastic gland seems to fail to respond to the stimulation with endogenous corticotrophin which is released during this test (12, 8) In non-endocrine disorders we have found plasma 17-OHCS during the pyrogen test to be an average of 75 of the increase in the same subjects during our standard ACTH test. In our case of Cushing's syndrome the response to the pyrogen test was within normal limits despite the supranormal response to ACTH This finding is in accordance with the observations of Shuster and Flynn (18) The experimental results of Wexler et al. (25) suggest that endogenous circulating corticoids are able to block the pituitary-stimulating effect of a pyrogen.

Summary

Corticotrophin secretion is controlled by two different mechanisms the feed-back (e. g metopiron) and the neurohumoral (e. g pyrogen) These two mechanisms are evaluated in 25 patients with pituitary and adrenocortical disorders. In the metopiron test the mediating stimulus is diminished circulating levels of cortisol, while the pyrogen test represents a stressful stimulus. The response to these pituitary function tests is measured by the increase in the urinary and plasma 17 hydroxycorticosteroids.

The metopiron test acting through a negative feed-back mechanism, appears to be the most sensitive measure available for the detection of defects in ACTH secreting efficiency

The pyrogen test is a very simple method for demonstrating inadequate ACTH release and less time-consuming Since it operates through a stressful stimulus, it is suggested that this will probably be particularly appropriate as a preoperative screening test in steroid treated patients. How reliable the test might be in predicting the pituitary-adrenal response to surgery remains to be seen.

Acknowledgements

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One-sided Kidney Affections and Arterial Hypertension

By

Erik Ask Upmark

Arterial hypertension may convenient ly be divided into one cryptogenic and one symptomatic group. The cryptogenic hypertension corresponds to the old word "essential hypertension" It represents some 75—80 % of all instances of arterial hypertension. There is reason to believe that constitutional factors underlie this type of hypertension. There is also some evidence that the supply of sodium may be of importance.

The symptomatic hypertension is sec ondary to certain affections of the cardio-vascular system, of the nervous system, of the suprarenals, of the kidneys and of the genital system. All taken together this group of arterial hypertension seems to represent at least 20—25 % of all instances. There are reasons to believe that this symptomatic hypertension with augmented medical knowledge will increase at the cost of the cryptogenic type.

In this paper only the renal hypertension will be considered. It may be due to a lesion of both kidneys, such as acute glomerulonephritis, periarteritis nodosa, polycystic kidneys or nephrosclerosis (due to chronic glomerulonephritis, to arterial hypertension per se, to chronic pyelonephritis or to damage through radiation or through abuse of phenacetin)

It may however be due also to an affection of one kidney a type of arterial hypertension which it is particularly important not to overlook, since such instances are amenable to surgery This cause of arterial hypertension has been dealt with repeatedly in the past, and the present paper represents a survey of our knowledge so far assembled.

Evolution of our knowledge

1 Anatomy the first demonstration of arterial hypertension in connection with a one-sided lesion of the kidney was made in 1929 by Ask-Upmark (1) References are to be had in the important papers of Poutasse (14) of Werkö and Bucht (15) of Perloff et al. (13) and of Yates-Bell (16)

2. Experimentally the observations of Goldblatt in 1934 (9) represent fundamental evidence. Although heralded by Hartwich (10) and by Chanutin and

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instances. The same holds for trophy of the ovaries.

ad (8) The presence of naevus vasculosus flammeus in the kidney region should raise suspicions about the vascular conditions of the kidney

ad (9). The different size of both kidneys in connection with malignant hypertension was observed by myself already in 1929 and attention was also called to this in another publication of mine (1 4)

ad (10) Particularly if atherosclerosis is the arterial affliction concerned, the presence of other vascular obstructions (femoral, coronary carotid) may be indicative.

ad (11) Whereas in males hypertrophy and eventually dilatation of the heart is likely to cause in most instances of arterial hypertension, this is for unknown reasons not the case with the female heart. There are exceptions, however one is renal hypertension (due to bilateral or to unilateral cause) another is longstanding phaeochromocytoma, and still another is the Conn-tumour (aldosteronom)

The following investigations are more or less necessary in every case suspected to represent renal hypertension

A. Ophthalmoscopy

B. Chest-examination by X rays (heart size, appearance of thoracic aorta)

C. Laboratory examination of kidneys (NPN xantoprotein, creatinine urine concentration bility)

D. Roentgenological survey of the abdomen and the kidneys.

E. Urography if necessary completed by pyelography

F. Abdominal aortography

H. Radioactive renography (I n hippuric compounds)

I. Bilateral nalysis of the urine of the ureters.

J. Aldosterone t be looked for in the urine

K. Surgical exploration and in this connection biopsy



Fig. 1. Diagram of the malformation described by Ask-Upmark in 1929 Three macroscopical features are characteristic different size of the kidneys, the presence of "blind" calix from the pelvis, approaching the surface of the kidney and, finally an indentation of the surface against the blind calix. The tissue situated between the surface of the kidney and the blind calix (dotted area) has three characteristic microscopical features, firstly absence of glomeruli, secondly distension of the convolutes so as to imitate colloid goiter and, thirdly alterations of the arteries (thickening of the walls, etc.). This malformation has positive correlation with malignant hypertension and may be identified clinically I has in itself nothing to do with pyelonephritis, although all malformations of the kidneys may favor the development of this disorder

Whereas (A) (B) (C) and (D) are mandatory in every case, the functional condition of the kidney will have to determine whether the other examinations may be carried out. As for (H) we have no sufficient information assembled so far although a valuable paper has been published recently by Magnusson. As for (I) we feel that the danger of complications so far excludes this method except in certain special cases. As to (K) our opinion strongly suggests that surgical exploration is to be preferred to puncture biopsies, the dangers of the last mentioned methods having been pointed out recently by Jönsson and Boijsen (12) and by Boijsen and Köhler (5)

Ferris (8) Goldblatt should be looked upon as a pioneer for our understanding of these difficult and entangled questions.

3 Clinically the very first paper dealing with arterial hypertension in connection with interference with the renal arterialisation was the thesis published by Bergendal in 1936 (5) One year later Butler (7) published his well known case of hypertension cured by nephrectomy

Anatomy of one-sided lesions of the kidney with hypertension

1 Malformation either a simple hypoplasia or the syndrome described in 1929 by myself (fig 1)

2 Arterial affections atherosclerosis, fibromuscular hyperplasia, preformed conditions such as aneurysms of the renal artery or abdominal coarctation of the aorta embolies, and various other conditions (such as blocking of an accessory artery to the kidney by the ureter certain types of arteritis, as described for instance by Danaraj aneurysma dissecans of the aorta etc.)

3 Pyelonephritis, recognized as important ever since the fundamental observations of Longcope. Already around the shift of the century Runeberg, of Finland called attention to the predilection of pyelonephritis for kidneys presenting a malformation. This may explain why several instances of malformations in the literature have been termed "pyelonephritis" one has simply overlooked the underlying malformation.

4 Various other conditions, such as a cicatricial perinephritis, which may be considered as the clinical equivalent of the cellophan-sac of Page and Corcoran

Suspicion of a one-sided kidney affection

should be raised in any case of arterial hypertension fulfilling one or several of the following criteria

1 Young age.

2 No familiar occurrence of hypertension

3 Abrupt onset.

4 Rapid malignant course.

5 Attacks in the history of loin pains with fever

6 Bruit over the renal artery

7 Hypogenitality

8 Naevus vasculosus in the kidney region

9 Kidneys differing in size

10 Presence of other vascular obstructions.

11 In females, enlargement of the heart.

Some few comments may be added

ad (1) This is not necessary Even cases aged 50 or more may have a one-sided kidney lesion.

ad (2) The positive occurrence of arterial hypertension in the family does not rule out a one-sided kidney affection.

ad (3) Obviously it is not always easy to date the start and the onset of hypertension. One may have to make with the information yielded by the history

ad (4) This feature is nearly always present, although it is usually to be judged only by repeated examinations.

ad (5) Such attacks may represent pyelonephritis but also other afflictions, such as arterial embolies, where fever is the rule rather than the exception and where hematuria usually is to be noted

ad (6) This bruit should be listened to from the ventral side (above and lateral to the umbilicus) as well as from the dorsal side (costovertebral angulus) When found it is conclusive evidence of an arterial affection.

ad (7) Cryptorchism may call the attention to the genitourinary system also in these



Fig. 5. Case 2. Abdominal aortography. Most of the right kidney has an ordinary arterial architecture, only the lower pole seems to be at dis-advantage as to the arterial supply.



Fig. 6. Case 2. Late phase of arteriography (nephrography). In accord with the impression obtained from fig. 5, the lower pole is obviously less well marked. I turned out at the operation to be supplied by an accessory renal artery from the aorta. This accessory renal artery crossed with the ureter but instead of developing hydronephrosis the artery became obstructed from the compression of the ureter. Resection of lower third of right kidney cured the hypertension.

The difference will have to be explained by the indications for abdominal aortography.

Two instructive cases out of our material may be allowed to illustrate the matter here in question.

Case 1. Janitor aged 67. When 55 auricular fibrillation, since continued. When aged 59, 63 and 66 cerebral embolies with transitory symptoms. A moderate hypertension has been known to exist since the age of 63. When 67 abruptly increased hypertension (to 280/160) with rather severe symptoms.

Aortography: left renal artery occluded.

Surgery (some 2 months after onset of the severe hypertension): the left kidney was removed. There was an organized embolus in the main renal artery but a small accessory renal artery had provided the kidney with at least some modest amount of blood.

Recovery after nephrectomy: BP 140/80.

Death 20 months later in a new cerebral embolus.

The right renal artery did present some degree of arteriosclerosis but it was on the whole viable. There was a small infarction in the right kidney which had undergone a compensatory enlargement.

Case 2. Mother of 3, aged 41. For two years rather severe hypertension. Aortography: lower pole of right kidney insufficiently vascularized by the arterial supply.

Surgery: an accessory artery to the lower pole of the right kidney had become obliterated by the pressure of the ureter whilst no hydronephrosis was present. Resection of the lower pole of right kidney. Complete recovery. BP afterwards 140/85.

The first of these cases demonstrates the fact that even in old individuals hypertension may be caused by a monorenal factor and relieved by appropriate



Fig 2. Case 1 Urography shows normal picture on the right side, silence on the left.



Fig. 4 Case 1 The patient lived for 20 months with normal blood pressure after his left-sided nephrectomy At the post mortem the renal artery on the right side (probe introduced) turned out to be entirely ·able.



Fig 3. Case 1 Abdominal aortography No left renal artery is to be seen although some vascular structures in the region of the left kidney suggest that it is not entirely devoid of circulation. The obstruction of th left renal artery turned out to be due to an organized embolus, whereas the scanty supply of this kidney was by virtue of an accessory artery

In agreement with Poutasse and Page it is our impression that the most common type of disorder responsible for arterial hypertension of monorenal origin is the affliction of the arteries, whereas pyelonephritis and malformations range second and third. In order to elucidate the occurrence of lesions of the renal arteries in arterial hypertension, we have during the last 3 years had 82 instances examined by our department of roentgenology (Head Professor Folke Knutsson) It turned out that in no less than 25 of these instances a positive observation was to be registered. This figure is larger than that obtained by Peart and Brown and co-workers at St. Mary's in London but smaller than that obtained by Perloff and her collaborators in San Francisco not to speak of the Cleveland material as represented by Poutasse and Page.

Peart Brown et al. 10 positive instances out of 160

Perloff et al. 70 positive instances out of 110

Poutasse and Page 131 positive instances out of 427

Ask Upmark 25 positive instances out of 82



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Acta Medica Scandinavica. Vol. 173, fasc. 2, 1963

From the IV Department of Medicine, University of Helsinki, Finland

Symptoms in Carriers of Diphyllobothrium Latum and in Non-infected Controls

By

Matti Saarni, Wolmar Nyberg, Ralph Gräsbeck
and Bertel von Bonsdorff

The symptoms appearing in persons harboring *diphyllobothrium latum* are largely unknown. The best known feature of tapeworm infection is tapeworm anemia, which is hematologically identical with genuine pernicious anemia and is observed in about two per cent of the carriers. Although the majority of the worm carriers do not suffer from anemia more than fifty per cent have a pathologically low serum vitamin B_{12} concentration, below 100 $\mu\mu$g/ml (10). The clinical importance of this latent deficiency is uncertain. Also, it may be assumed that the worm causes harmful effects in other ways, but information on this subject is contradictory.

The opinion of several authors is that the majority of fish tapeworm carriers enjoy perfect general health (3, 7, 9, 11). According to Becker (1) most worm carriers suffer from fatigue quite unrelated to the intensity of the hematologic changes. He states that weakness, dizziness and headache are common symptoms amongst non-anemic carriers. Gastrointestinal symptoms have been mentioned most often. Stomatitis and glossitis have been observed among the worm carriers, and burning and stinging painful sensations in the oral mucosa are considered to be one of the most frequent symptoms of the worm infection. It has been stressed that this complaint may be so mild that patients do not spontaneously mention it. Even severe chronic stomatitis has been observed (2, 6). In the morning there may be a bad taste in the mouth. Epigastric fullness and pain following ingestion of food are often mentioned (2, 3, 7, 9). It is stated that the worm carrier has abdominal discomfort and a sensation of pressure or of strange movements especially in the umbilical region, but even severe abdominal pain simulating perforation of a peptic ulcer, biliary colic or intestinal obstruction have been reported. It is generally believed that poor appetite or lack of appetite

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surgery The presence of a small accessory artery did provide some circulation in the afflicted kidney had there been none whatsoever no hypertension ought to have ensued

The second case may serve as an (inverted) illustration to the pioneer work of Bergendal on the appearance of hypertension in connection with obliteration of accessory arteries of the kidney In Bergendal s thesis the arteries had caused a certain block to the drainage of the urine, and were hence to be sacrificed (with ensuing hypertension), whereas in this case of mine the ureter was the culprit and the artery had become blocked

Summary

1 A survey is given of our present knowledge about arterial hypertension as caused by affection of one kidney only

2 Among 82 instances of arterial hypertension examined by means of abdominal aortography there were 25 in whom an affliction of the renal artery was to be registered

3 Two cases are quoted as an illustration one of a man aged 67 who got a severe hypertension after the partial blocking of the renal arterial supply by an embolus, one of a female, aged 41 who had got an obliteration of an accessory renal artery to the lower pole of the right kidney In both instances recovery ensued after nephrectomy and renal resection respectively

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age groups (19—30, 31—60, and 61—90 years). The significance of the differences between the occurrence of various symptoms in the tapeworm ova + and — groups was tested using 2 × 2 table analysis. Adjusted χ^2 was calculated by means of an electronic computer (IBM calculator 602 A).

Results

1,345 persons or about 80 per cent of the subjects to whom questionnaire forms had been distributed visited the reception offices. 168 incompletely filled-in forms were discarded. 50 anemic cases were not included in the material. Thus the present material consists of 1 127 non-anemic subjects. Of these 295 belong to the tapeworm ova + group and 832 to the tapeworm ova — group. The answers to the question "are you at the present moment infected with the fish tapeworm?" were as follows: tapeworm ova + group "don't know" 60 per cent, "no" 3 per cent, "yes" 37 per cent. Tapeworm ova — group "don't know" 87 per cent, "no" 8 per cent, "yes" 5 per cent.

The question regarding the subject's own opinion on the symptoms caused by the tapeworm was answered by 455 persons. The most popular belief was that the tapeworm produces abdominal pains. Secondly dizziness, fatigue and weakness were thought to accompany the infection. 55 persons considered hunger and only 3 poor appetite as a symptom. Special attention has to be drawn to the curious "craving for salt" which will be discussed later. This symptom is mentioned by 42 subjects. Soreness of the mouth, regarded as a main sign of worm infection in some worm districts, was mentioned only by 9 persons. In addition to the symptoms seen in table I there are occasional statements of strange sensations in various organs.

Table I. The popular opinion concerning the symptoms caused by the fish tapeworm. 455 cases

Symptoms	No.	%
Abdominal pains	143	31
Dizziness	100	22
Fatigue, weakness	93	20
Sensation of hunger	55	12
Craving for salt	42	9
Vomiting	33	7
Diarrhea	30	7
Epigastric fullness	21	5
Headache	22	5
Flatulence	17	4
Soreness of mouth	9	2
Heartburn	5	1
Lack of appetite	3	1

The answers given to the 4th main point in the questionnaire form were subjected to statistical analysis. The results are seen in table II. The persons without worm infection are healthier, i. e. more often have no symptoms whatsoever than the worm carriers (highly significant) although as many as 44 per cent of the former group stated that they were not in perfect condition. The most significant difference between the groups was seen in the symptom "craving for salt". For this expression there is a special local term "himkominen" which implies almost salt addiction. Closer questioning regarding this phenomenon revealed that the salt hunger appears suddenly and unexpectedly and is accompanied by a feeling of weakness. It disappears very quickly after eating salt, salted or raw fish. The worm carriers suffer more from fatigue, dizziness and weakness than the control group (highly significant). Of the gastrointestinal symptoms, diarrhea is found more frequently in the worm carrier group (highly significant). In this group, the sensation of hunger is also

for certain foods accompanies infection with the fish tapeworm. Schauman however in his study of tapeworm anemia states that poor appetite appears only in connection with comparatively severe anemia (12) The worm carriers may have nausea and tapeworm vomiting occurs rarely This last phenomenon is often associated with collapse, severe pain and feeling of suffocation (5) Most investigators have observed diarrhea as a common symptom This diarrhea is usually painless (2, 3 7 9 11) On the other hand Becker mentions constipation alternating with diarrhea (2) A chronic diarrhea is always indicative of other diseases (7) Many neurological and psychical manifestations have been described epileptiform and hysterical crises choreiform phenomena, convulsive states, paralysis, strabismus, aphonia, dyspneic attacks, visual disturbances and pruritus of the nose and anus (3) No attention has been paid to the incidence of the symptoms in earlier investigations The differences in the opinions are probably due to failure to distinguish between manifestations of tapeworm anemia and the other symptoms caused by the presence of the parasite and observable in non anemic worm carriers. Indeed no systematic study of the symptoms of non anemic worm carriers has been performed. We therefore considered it important to obtain a more accurate picture of the symptomatology in non-anemic worm carriers in Finland where the incidence of the fish tapeworm infection is high.

Materials and methods

This investigation is a part of a large field study in Eastern Finland (8, 10) Before the investigation, questionnaire forms were distributed to the school children in all the schools in the area. The pupils received a questionnaire form for every member of their family On the same occasion the purpose of the investigation was explained

The main points in the questionnaire form concerning this investigation were as follows

1 Name. Age. Birth date. Occupation. Address.

2 Which are the symptoms caused by the tapeworm in your opinion?

3. Are you infected with the tapeworm at the moment of investigation and why do you think so?

4 A detailed list of questions dealing with generally accepted tapeworm symptoms together with control questions about other common disease symptoms never yet mentioned in connection with tapeworm infection. The following symptoms were mentioned in our questionnaire form hunger sensation, loss of weight fatigue, weakness, dizziness, diarrhea, constipation, flatulence, abdominal pains, heartburn, salt craving, visual disturbances, antipathy to seeing certain things, figures or colors, impaired vision in darkness, weakness of the eyes, swelling around the eyes, black rings around the eyes, headache, darkened vision, palpitation, chest pains, dyspnea, hair turning gray fever soreness of the tongue, gingiva, and the corners of the mouth, itching, rash, brittle nails, pallor bleeding from the nose, numbness in the extremities, formication, manual clumsiness, reduced sensibility palsy The symptoms were expressed in lay terms and most questions were in the form do you often suffer from this trouble?" Every question had to be answered by "yes" or "no" but many subjects provided modified answers. Because of the general nature of the tapeworm symptomatology vague answers like sometimes "probably" etc which were sometimes given were regarded as positive in the analysis.

The filled-in forms were delivered at temporary reception offices. On the same occasion the feces were examined for tapeworm ova and blood samples taken for hematologic studies and vitamin B_{12} determination. The results of the laboratory findings have been published (8, 10) Incompletely filled-in forms were eliminated. *All anemic subjects were also excluded* (Hb < 11 0 g%) The series was divided into two main groups tapeworm ova present (+) and tapeworm ova absent (—) These were further divided into sex and

age groups (19—30, 31—60, and 61—90 years). The significance of the differences between the occurrence of various symptoms in the tapeworm ova + and — groups was tested using 2 × 2 table analyses. Adjusted χ^2 was calculated by means of an electronic computer (IBM calculator 602 A).

Results

1,345 persons or about 80 per cent of the subjects to whom questionnaire forms had been distributed visited the reception offices. 168 incompletely filled-in forms were discarded. 50 anemic cases were not included in the material. Thus the present material consists of 1 127 non-anemic subjects. Of these 295 belong to the tapeworm ova + group and 832 to the tapeworm ova — group. The answers to the question "are you at the present moment infected with the fish tapeworm?" were as follows: tapeworm ova + group: don't know 60 per cent, no 3 per cent, yes 37 per cent. Tapeworm ova — group: don't know 87 per cent, no 8 per cent, yes 5 per cent.

The question regarding the subject's own opinion on the symptoms caused by the tapeworm was answered by 455 persons. The most popular belief was that the tapeworm produces abdominal pains. Secondly dizziness, fatigue and weakness were thought to accompany the infection. 55 persons considered hunger and only 3 poor appetite as a symptom. Special attention has to be drawn to the curious "craving for salt" which will be discussed later. This symptom is mentioned by 42 subjects. Soreness of the mouth regarded as a main sign of worm infection in some worm districts, was mentioned only by 9 persons. In addition to the symptoms seen in table I there are occasional statements of strange

Table I. The popular opinion concerning the symptoms caused by the fish tapeworm. 455 cases

Symptoms	No.	
Abdominal pains	143	31
Dizziness	100	22
Fatigue weakness	93	20
Sensation of hunger	55	12
Craving for salt	42	9
Vomiting	33	7
Diarrhea	30	7
Epigastric fullness	21	5
Headache	22	5
Flatulence	17	4
Soreness of mouth	9	2
Heartburn	5	1
Lack of appetite	3	1

The answers given to the 4th main point in the questionnaire form were subjected to statistical analysis. The results are seen in table II. The persons without worm infection are healthier, i.e. more often have no symptoms whatsoever than the worm carriers (highly significant) although as many as 44 per cent of the former group stated that they were not in perfect condition. The most significant difference between the groups was seen in the symptom "craving for salt". For this expression there is a special local term "hnukommen" which implies almost salt addiction. Closer questioning regarding this phenomenon revealed that the salt hunger appears suddenly and unexpectedly and is accompanied by a feeling of weakness. It disappears very quickly after eating salt, salted or raw fish. The worm carriers suffer more from fatigue, dizziness and weakness than the control group (highly significant). Of the gastrointestinal symptoms, diarrhea is found more frequently in the worm carrier group (highly significant). In this

Table II Incidence of symptoms in non-anemic worm carriers and control subjects

Symptoms	Worm carriers		Control subjects		Comparison of groups	
	No.	%	No.	%	χ^2	p <
Not perfectly healthy	174	59.0	363	43.6	19.97	0.001
Craving for salt.	183	62.0	346	41.6	35.74	0.001
Diarrhea	65	22.0	82	9.9	27.41	0.001
Fatigue and weakness	194	65.8	413	49.6	22.14	0.001
Dizziness	156	52.9	321	38.6	17.66	0.001
Numbness of the extremities	145	49.2	311	37.4	12.05	0.001
Sensation of hunger	109	36.9	226	27.2	9.52	0.01
Reduced sensibility	24	8.1	34	4.1	6.51	0.05
Headache	126	42.7	286	34.4	6.17	0.05
Impaired vision in darkness	158	53.6	388	46.6	3.91	0.05
Sore tongue.	51	17.3	105	12.6	3.60	—
Abdominal pains	133	45.1	331	39.8	2.31	—
Heartburn	96	32.5	238	28.6	1.44	—
Formication	41	13.9	94	11.3	1.16	—
Sore gums	38	12.9	87	10.5	1.06	—
Symptoms resulting from incoordination and disturbances of motor function	32	10.8	79	9.5	0.31	—
Flatulence	113	38.3	312	37.5	0.03	—
Soreness of the corners of the mouth	38	12.9	101	12.1	0.05	—
Constipation	77	26.1	234	28.1	0.35	—
Loss of weight	18	6.1	65	7.8	0.70	—

more prominent (significant) The only neurological symptom which occurs more frequently in the tapeworm ova + group is numbness of the extremities (highly significant) Contrary to what had been expected there was no significant difference between the groups in the incidence of abdominal pains. The same is true regarding the symptoms from the oral mucosa. Also worth mentioning is the fact that constipation and loss of weight occur somewhat more frequently in the control group This difference however is not statistically significant

The difference between the sexes

The material consisted of 681 men and 446 women The male worm carriers show a higher incidence of diarrhea than the male group without tapeworm ova in the stools (31.5 % and 10 3 % χ^2 = 28 16 $p < 0.001$) On the other hand, there was no difference in this respect between the female tapeworm ova + and — groups (14 9 % 9 6 % χ^2 = 3 18) Among the females the sensation of hunger was more prominent in the tapeworm ova + group than in the — group (43 5 % 26 9 % χ^2 = 15 45 $p < 0.001$) whereas no difference in this respect was seen in the male groups (ova + 28.3 % ova — 27 6 %) Other significant differences between the sex groups could not be found, although the females show a slightly higher incidence of dizziness, tiredness headache and constipation.

The difference between the age groups

The material was divided into three age groups (table III) The relative num-

Table III Incidence of symptoms in non-anemic worm carriers and control subjects in three age groups

Symptoms	9—30 years		31—60 years		61—90 years	
	Worm carriers (no. 112)	Control subjects (no. 502)	Worm carriers (no. 144)	Control subjects (no. 274)	Worm carriers (no. 39)	Control subjects (no. 56)
Not perfectly healthy	43 38%	130 26%	103 71%	192 70%	28 72%	41 73%
Craving for salt	59 53%	155 31%	101 70%	164 60%	13 33%	27 48%
Diarrhea	22 20%	41 8%	38 26%	36 13	5 13	5 9%
Fatigue, weakness	62 55%	197 39%	105 73%	178 65%	27 69%	38 68%
Dizziness.	43 38%	145 29%	85 59%	145 53%	28 72%	31 55%
Sensation of hunger	49 44	133 27%	46 32%	81 30%	14 36%	12 21%
Headache	41 37%	130 26%	71 49%	140 51%	14 36%	26 46%
Abdominal pains	46 41%	162 32%	70 49%	145 53%	17 44%	24 43%
Heartburn	35 31%	118 24%	52 36%	95 35%	9 23%	25 45%
Flatulence	34 30%	140 28%	59 41%	142 52%	20 51%	30 54%
Constipation	19 17%	95 19%	38 26%	107 39%	20 51%	32 57%
Loss of weight	5 5%	34 7%	7 5%	20 12%	6 15%	11 20%

Question Are you perfectly healthy

ber of worm carriers increases with increasing age. As expected there was a sharp increase in the incidence of symptoms in the older groups. Regardless of age, the worm carriers were more tired, suffered more from dizziness and weakness than the subjects in the control group Between the ages of 9 to 30 years the worm carriers suffered more from headache than the non-carriers ($p < 0.05$) From 31 years upward there was no difference in the frequency between the tapeworm + and — groups. There is also a significantly higher incidence of diarrhea among the worm-infected persons than among the non-infected ones in the age groups 9—30 and 31—60 years ($p < 0.001$ and $p < 0.01$) At all ages, the sensation of hunger was more prominent in the worm carriers than in the tapeworm ova — group but the difference was significant only in the youngest group.

Table II Comparison of symptoms in non-anemic worm carriers with normal and pathologically low serum vitamin B_{12} concentration

Symptoms	$B_{12} \leqq 100$ $\mu\mu$g/ml 152 cases		$B_{12} > 100$ $\mu\mu$g/ml 143 cases		Comparison of groups	
	No	%	No.	%	χ^2	p <
Dizziness	93	61	63	44	8.00	0.01
Neurological symptoms	78	51	55	39	4.41	0.05
Fatigue, weakness	105	69	89	62	1.24	—
Craving for salt	97	64	86	60	0.28	—
Sensation of hunger	50	33	49	34	0.02	—
Not perfectly healthy	90	59	84	59	0.00	—

Symptomatology in tapeworm carriers in relation to the serum vitamin B level

The 295 worm carriers were divided into two groups one with a serum B_{12} level $\leqq$ 100 $\mu\mu$g/ml and the other with a value > 100 $\mu\mu$g/ml Low values were found in 152 cases and normal B_{12} concentration in 143 cases. Only the symptoms for which a highly significant difference between the tapeworm ova + and ova — group was found were subjected to analysis (table IV) It is seen from the table that no correlation with low serum B_{12} levels is noticed with the exception of dizziness and other neurological symptoms.

Comments

This mass examination gave us the opportunity to collect and analyze the subjective complaints of non anemic fish tapeworm carriers and to compare them with those of a control group consisting of non-infected persons. It has to be stressed that, in contrast to earlier investigations, this material consists not of hospitalized subjects but of people fully fit for work. The opinions of the population of this "tapeworm district" concerning the symptomatology are of interest. They differ somewhat — but not as much as those of earlier authors — from the results obtained by the statistical analysis (tables I and II) For instance, there is no statistical evidence that the worm carriers have more abdominal pains than the non-carriers. On the other hand it is quite natural that laymen associate an intestinal parasitic infection with abdominal pains.

The high incidence of complaints in the control group is partly due to the provocative "pitfall" questions included in the forms. It may be suspected that the consciousness of being a worm carrier would influence the answers. However 37 per cent of the worm carriers were aware of a present worm infection and only 8 per cent of non-infected persons were sure that they had no infection. It is obvious that the difference between the groups cannot be explained on this basis. Furthermore, against such an explanation stands the fact that the popular belief about the symptoms caused by the worm are different from those obtained in this investigation (tables I and II) On the other hand it is known that in a small percentage of worm carriers no ova are found in the stools (7) In our

earlier statistical investigation of the present material we obtained the over-all impression that not more than 5 to 10 per cent of the tapeworm ova negative group were worm carriers" (8)

The sensation of salt hunger is an interesting symptom, which has not been described earlier It is possible that we are dealing with a symptom which has nothing to do with the worm, but that persons having salt craving are especially fond of raw or salt fish. Against this speaks the fact that this symptom often disappears after the worm cure. This topic needs further investigation. It has been shown that fatigue dizziness and weakness very often accompany the infection with the fish tapeworm. With the exception of Becker (1) very few authors have drawn attention to these general symptoms. The sensation of hunger seems to be characteristic for the fish tapeworm infection, and it is probable that the often mentioned lack of appetite and loss of weight become prominent only after the anemia has developed, as pointed out by Schauman (12) Furthermore, we have not been able to verify that changes in the oral mucosa are typical for uncomplicated tapeworm infection. Probably this symptom, too, is a feature of tapeworm anemia. Of the numerous neurologic symptoms included in the forms only *numbness* of the limbs seems to occur significantly more often in the tapeworm ova + group. This is in good accordance with Björkenheim objective findings (4)

Diarrhea and increased appetite are the only symptoms where differences are seen between the sexes. With the exception of vertigo no clearly significant correlation between the serum vitamin B_{12} level and the incidence of symptoms has been shown. A closer correlation should be expected. However the tapeworm carrier material is relatively small and dominated by the youngest age group with a relatively high mean serum B_{12} level 141 µµg/ml despite worm infection, and this may mask the true correlation.

The large control group in our material has made it possible to analyze the true incidence of even mild and vague symptoms said to be connected with tapeworm infection. *The results show how misleading the frequency of such symptoms, reported without reference to a control group can be.* For instance, 45 per cent of the worm carriers had abdominal pains. This is in good accordance with the earlier conception (1) However as many as 40 per cent of the non-infected control group also had abdominal pains. This point is quite important and seems to be of a more general interest. The symptom frequencies generally found in clinical reports are of limited value when the corresponding values in a control group are not given. If we assume that the tapeworm infects a population the percentage of the incidence of symptoms (i) *caused by the worm* may be calculated as follows

$$i = \frac{(t - c)\ 100}{100 - c}$$

where t = per cent incidence of symptoms in tapeworm carriers and c = per cent incidence of symptoms in controls. Using this equation we find the "true" incidence of symptoms caused by the worm to be as follows craving for salt 35 %, fatigue and weakness 32 %, dizziness 23 %, numbness of the extremities 19 %, diarrhea 13 %.

With the exception of the tapeworm anemia, the symptomatology of *diphyllobothrium latum* infection is without char

Table IV *Comparison of symptoms in non-anemic worm carriers with normal and pathologically low serum vitamin B_{12} concentration*

Symptoms	$B_{12} \leqq 100$ $\mu\mu g$/ml 152 cases		$B_{12} > 100$ $\mu\mu g$/ml 143 cases		Comparison of groups	
	No	%	No.	%	χ^2	p <
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Fatigue weakness	105	69	89	62	1.24	—
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Sensation of hunger	50	33	49	34	0.02	—
Not perfectly healthy	90	59	84	59	0.00	—

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Combined Guanethidine and Hydrochlorothiazide Therapy in Hypertension

By

A. M. Abrahamsen, S. Humerfelt and H. Sigstad

Maxwell and his co-workers (22 23) have shown that guanethidine has a protracted blocking effect on the post ganglionic sympathetic fibres, and at the same time hardly any effect on the parasympathetic nervous system. The blocking probably occurs in the peripheral branches of the postganglionic fibres, presumably by reducing the liberation of catecholamines.

It is said that the fall in blood pressure is due to a reduction in the cardiac output, probably because the lack of sympathetic [v]enous constriction results in pooling of the blood in the peripheral [ve]ins thus reducing the venous return (26 27).

The mechanism of the anti-hypertensive effect of the thiazide derivatives is not quite clear.

Chlorothiazide increases the excretion of sodium, chlorides and water and because of this a reduction in the extra cellular fl[u]id and in the plasma volume follows (4 7 9 10 13, 31). This leads to a reduction in the filling pressure in the right atrium and consequently to a reduction in the cardiac output. In short term treatment this appears to be the direct cause of the fall in blood pressure (4 7 11 35). Reduction in the blood volume after a few days treatment has been seen with chlorothiazide and with hydrochlorothiazide (28).

Some hold that, particularly during long term treatment, the effect is due to a reduction in the sodium content of the arterial walls (29 33). It is possible that the thiazide derivatives reduce the activity of an unknown pressor agent present in hypertension (15 34).

Many trials have shown that [i]t is useful to combine guanethidine with a diuretic of the thiazide group (3 12, 14 16). It has then often been possible to reduce the dose of guanethidine considerably.

The purpose of our investigation has been to find out how much the hypotensive effect of guanethidine is accentuated when hydrochlorothiazide is used at the same time.

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probably partly caused by the over all action of the parasite on the nutritional state of the host partly by the local effect on the intestine and perhaps by the absorption of metabolic products originating in the worm.

Summary

Little was known concerning the symptoms found in non-anemic fish tapeworm carriers and contradictory statements are found in the literature In a large field study 1 127 persons (295 worm carriers 832 non infected subjects) were questioned regarding their subjective state of health Statistical analysis showed that compared to the controls the worm carriers had a very significantly higher incidence ($p < 0.001$) of fatigue, weakness dizziness diarrhea and numbness of the extremities. Also sensation of hunger was more frequent among the worm carriers ($p < 0.01$) No difference in the incidence of abdominal pains and symptoms from the oral mucosa between the groups was found A symptom "craving for salt not earlier mentioned was found to be correlated with worm infection Persons under 30 years of age had more symptoms from their worm infection than older worm carriers. With the exception of dizziness no clearcut correlation between the symptoms caused by the worm and the serum vitamin B_1 levels could be found in the non anemic subjects. Some of the symptoms earlier believed to be caused by the tapeworm were found to be unrelated to the infection This stresses the importance of including controls when the frequency of vague clinical symptoms is studied.

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Table II Effect of intravenous guanethidine on recumbent arterial pressure

Patient, Sex	Age	Guanethidine Before M.A.P (mm Hg)	Guanethidine After M.A.P (mm Hg)	Reduction in M.A.P (%)	Time of maximum effect (min)
J T ♂	56	165	123	25	235
O. N. ♂	43	182	117	36	65
F Ö. ♂	46	181	117	35	300
L H. ♂	59	157	100	36	150
K. J. ♀	37	110	98	11	15
G. V ♀	65	150	90	40	105
M. P ♀	57	180	107	41	140
G. K. ♀	62	147	103	30	135
E. P	54	137	93	32	70
A. R. ♀	45	128	97	24	145
G. K. ♀	44	137	87	36	10
H. O. ♀	48	157	108	31	35
Average and standard deviation		152 6 ± 21 5	103.3 ± 6.0	31 4 ± 7.9	117 1 ± 26.4

M.A.P = mean arterial pressure (diastolic pressure plus ⅓ of the pulse pressure).

Table III Effect of intravenous guanethidine on recumbent arterial pressure after 3 day treatment with hydrochlorothiazide

Patient, Sex	Age	Guanethidine Before M.A.P (mm Hg)	Guanethidine After M.A.P (mm Hg)	Reduction in M.A.P (%)	Time of maximum effect (min)
J. T ♂	56	143	110	23	185
O N. ♂	43	143	110	23	310
F Ö. ♂	46	167	153	8	95
L H. ♂	58	157	87	44	155
K. J. ♀	37	127	115	9	165
G. V ♀	65	133	83	38	45
M. P ♀	57	197	73	63	25
G. K. ♀	62	165	92	44	75
E. P ♀	54	123	107	13	10
A. R. ♀	45	115	88	23	235
G. K. ♀	44	115	80	30	45
H. O. ♀	48	130	90	31	45
Average and standard deviation		142.9 ± 23.6	99.0 ± 20 8	29.1 ± 13.5	114.2 ± 90.4

M.A.P = mean arterial pressure (diastolic pressure plus ⅓ of the pulse pressure)

49 1/2 years. All had retinal changes K. W & B. I—IV mainly I Half of them had electrocardiographic changes indicative of left ventricular hypertrophy As judged from the X-ray investigations 7 patients showed signs of enlargement of the left ventricle. The heart volume, calculated according to Jonsell's formula was, in most cases, within the range of the normal variation, being on an average 416 ml/m In 1 man (F Ö) and 2 women

Table I Grouping of the series

Patient Sex	Age	K. W & B.	ECG	Heart vol. (ml/m²)	Prot-uria	Creatinine
J T ♂	56	I	Normal	460	−	1.3
O N ♂	43	III	L.V.H.	390	(+)−	1.5
F Ö ♂	46	III—IV	L.V.H.	760	+	1.3
L H. ♂	59	I	Normal	440	−	1.2
K. J ♀	37	I	Normal	380	−	1.2
G V ♀	65	I	L.V.H.	330	−	1.4
M. P ♀	57	III	L.V.H.	530	+ −	1.1
G. N ♀	62	I	Normal	380	−	1.4
E. P ♀	34	I	Normal	210	−	0.9
A. R. ♀	45	I	Normal	300	−	1.1
G K. ♀	44	I	L.V.H.	370	+	1.0
H. O ♀	48	III	L.V.H.	450	−	1.3

L.V. H. = left ventricular hypertrophy

Methods

The investigation was planned as a short term one using an intravenous infusion of guanethidine[1] given in the course of 15 min. The dose given was 0.25 mg/kg body weight dissolved in 100 ml 5 % glucose. This was given before and after 3 days oral treatment with hydrochlorothiazide,[1] 75 mg daily

Routine kidney function tests, X-ray investigations of the heart and urography in addition to electrocardiographic investigations and blood pressure measurements were carried out on all patients.

The patients were treated with bed rest and a low salt diet for several days before the trial. Twenty four hour specimens of urine were collected the day before the first guanethidine infusion and for the following 7 days. The blood pressure was measured by auscultation at regular short intervals before the infusion and during the first 3 1/2 hours following it. It was recorded every half hour during the next 3 hours and then every hour for a total of twelve hours in all.

The total amounts of Na⁺ K⁺ Cl⁻ and urea, uric acid and creatinine were measured in the 24 hour urine specimen. Before and after treatment with hydrochlorothiazide and before the guanethidine infusion, the serum was analysed for the same substances.

Guanethidine (Ismelin®) and hydrochlorothiazide (Esidrex®) were supplied through the courtesy of Ciba Ltd. (Kobro & Co Oslo)

In patient E. P seven 24 hour urine specimens were examined for the catecholamine metabolite 3-methoxy-4-hydroxymandelic acid (VMA) and in 9 patients the aldosterone content of the 24 hour specimen was investigated before and after treatment with hydrochlorothiazide

The plasma volume and the cardiac output were estimated by the dye dilution technique just before each of the guanethidine infusions. Evans blue was used and the concentration was recorded by means of a modified ear oxymeter

In both the urine and the serum the Na⁺ and the K⁺ were estimated using a Perkin Elmer flame photometer the Cl⁻ by potentiometric titration and the uric acid by Praetorius method (25) Van Slyke's method was used to estimate the urea in the urine and the serum and for the alkali reserve The serum protein was estimated by the Biuret method aldosterone by Neher and Wettstein's physicochemical method (24) The VMA was estimated by a combination of Armstrong and co-worker's paper chromatographic method (1) and von Studnitz's spectrophotometric method (30)

Material

The material consisted of 12 patients, 8 women and 4 men (table I) The age varied from 34 to 65 years with an average age of

marked difference in the fall in blood pressure before and after hydrochlorothiazide therapy Both these patients were psycholabile.

In three patients there was a short rise in the blood pressure after a 5—10 mm. latent period. This lasted from 5—10 min. In the one patient with malignant hypertension (F O) the blood pressure remained at this higher level for the following 35 min.

The effect on the blood pressure after both the infusions was relatively long lasting, being from 6 to 8 hours in most cases. In all cases the blood pressure remained lower on the days following the infusions than it had been during the control period before the infusions.

Seven patients had symptoms of postural hypotension for one to three days following the investigation.

The urinary output measured on the first infusion day was considerably lower than on the preceding control day the difference, 290 ml/24 hours is significant ($P < 0.001$) The electrolyte excretion in the same urine specimen showed that the potassium excretion had been reduced significantly on an average 8.2 mEq/24 hours ($P < 0.001$) while the Na and Cl excretion did not show any significant reduction.

In the 3 day period with hydrochlorothiazide therapy a marked increase in the urinary output occurred in comparison to that in the previous control period. In addition an average weight reduction of 1 7 kg was found. All the patients showed a significant increase in Na and Cl excretion as well as in K excretion ($P < 0.001$)

After the hydrochlorothiazide period the serum N concentration showed a slight, but insignificant drop from an erage of 139.5 mEq/l to 136 9 mEq/l

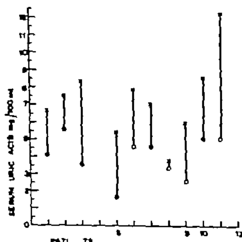


Fig 2 The increase in the serum uric acid after three days treatment with hydrochlorothiazide (75 mg/day) combined with guanethidine infusion.

— before hydrochlorothiazide.
× — after

compared with the serum concentration before the first guanethidine infusion. On the other hand, the K and Cl in the serum were significantly reduced averaging from 4 4 to 3.5 mEq/l and from 102 to 95 mEq/l, respectively ($P < 0.001$) In 4 patients there was a fall in the serum potassium 5 1 1 mEq/L Two patients (M. P and G V) showed a fall to 2.8 mEq/L In one patient (E. P) moderate electrocardiographic changes occurred and subjective signs of hypopotassaemia. At the same time the serum potassium sank from 4.9 mEq/l to 3.8 mEq/L

The alkali reserve increased in 8 out of 9 patients by an average of 2.8 mEq/l. This is a significant increase ($0.001 < P < 0.01$)

In 10 out of 11 patients investigated there was an increase in the serum creatinine from an average of 1.3 to 1.5 mg/100 ml. This increase is significant ($0.001 < P < 0.01$) The creatinine ex

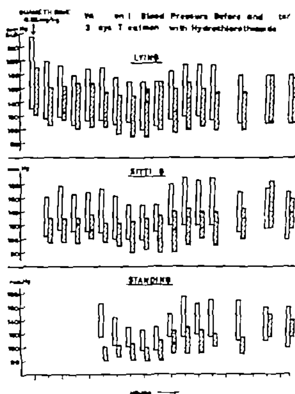


Fig 1 Blood pressure variations in patient J T The white columns represent the systolic and the diastolic blood pressures following the first intravenous infusion of guanethidine. The hatched columns represent the corresponding blood pressure readings following the second guanethidine infusion — after hydrochlorothiazide therapy on the three preceding days. (In the first 3 1/2 hours the mean values of three consecutive readings are given.)

□ = before hydrochlorothiazid

▢ = after

(M. P and H O) the heart volume was pathologically increased.

Four patients had proteinuria, and the serum creatinine was 1.5/100 ml in two of these. All of the eleven investigated showed negative findings on urography

Results

All the patients showed a considerable fall in the systolic and the diastolic blood pressure in connection with the guanethidine infusions. The effect was increased in the sitting position, and was even more marked on standing The pulse pressure became lower after the guanethidine infusion and even lower when this had been preceded by hydrochlorothiazide therapy

After the first guanethidine infusion (table II) the mean arterial pressure in the recumbent position fell from, on an average 152 6 mm Hg to 103.3 mm Hg with an average reduction of 31 4 %. The maximal effect came after an average of about 2 hours.

The starting pressure at the second guanethidine infusion, after 3 days of hydrochlorothiazide therapy was on an average 10 mm below the corresponding pressure at the first guanethidine infusion (before hydrochlorothiazide therapy) (table III) The average fall was from 142.9 mm Hg to 99 mm Hg with a mean reduction of 29 1 % The time taken to obtain the maximal effect averaged 2 hours here also.

The fall in blood pressure in the sitting position and standing increased after the second guanethidine infusion and only 3 of the patients were able to have all the blood pressure measurements taken in the standing position following hydrochlorothiazide therapy The remainder developed such severe symptoms of postural hypotension that it was thought unadvisable to take these measurements.

Fig 1 shows the variations in blood pressure in patient J T

The blood pressure measurements on the days of the infusion followed this pattern completely in 9 of the patients. One patient (F Ö) however showed higher blood pressure readings both before and during the second infusion but a fall in the systolic and the diastolic blood pressure followed in this patient also This patient had malignant hypertension Two patients (E. P and K J) showed no

After guanethidine-hydrochlorothiazide treatment the plasma volume fell in 6 patients, in one it remained unchanged and in 5 there was an increase in the plasma volume. The average reduction in plasma volume was 0 11 (not significant) Out of the 5 patients investigated there was a decrease in the cardiac output in 4 and an increase in one (fig 4)

The results of all the urine and serum investigations on patient J T are illustrated in fig 3

In one patient 7 24-hour urine specimens were investigated to find their content of the catecholamine metabolic 3-methoxy-4-hydroxy-mandelic acid. The results indicate that the general production of catecholamines is unchanged the values lay around the lower limit of the normal value.

The aldosterone excretion in 24 hour urine specimens was examined in 8 patients before and after the combined therapy In addition in one patient (E. P) it was estimated for the following 9 days. The results show that out of 25 specimens investigated the excretion was increased (21—45 mg) in 4 in 3 of which the increase occurred after the treatment had ended.

Side-effects

As mentioned above all the patients suffered from postural hypotension on both the infusion days, and in only three was it possible to carry out all the blood pressure readings with the patient standing

Seven patients had symptoms of postural hypotension on the three days between the first and second guanethidine infusions and on the day following the second infusion. Neither bradycardia nor diarrhoea were recorded.

Discussion

The investigations show that the intravenous infusion of guanethidine has a marked hypotensive effect in hypertension. The effect increases in the sitting position and is greatest on standing As others have shown there is an initial increase in pressure in some patients probably due to the immediate release of catecholamines from the sympathetic post ganglionic fibres (8, 20 22) This, and the fact that the maximum effect is first evident after about 2 hours, means that guanethidine is probably not really suitable for intravenous use in the treatment of hypertensive crises. After three days oral treatment with hydrochlorothiazide there is an increase in the effect of the same dose of guanethidine. The mechanism is probably the same as that in combined therapy with thiazide diuretics and ganglion blocking agents. Many (7 31 35) have stated that oligaemia increases the vasomotor tone, which again makes the patients more sensitive to ganglion blocking agents.

The theory that hydrochlorothiazide reduces the blood pressure by reducing the plasma volume, is not definitely supported by this investigation, although the plasma volume fell in most of the patients. The patients had, however also been given guanethidine which has a tendency to cause water retention (5 12, 14 16) The present investigations show that there was a marked reduction in the urinary output on the day of the first infusion, compared with that in the control period. At the same time there was a significant reduction in the K excretion while the Na and Cl excretion was not markedly reduced.

In this series guanethidine was given intravenously The effect must therefore

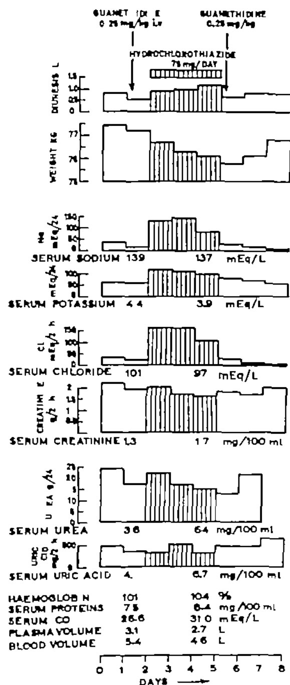


Fig 3. The effect of guanethidine combined with hydrochlorothiazid on patient J T (with essential hypertension)

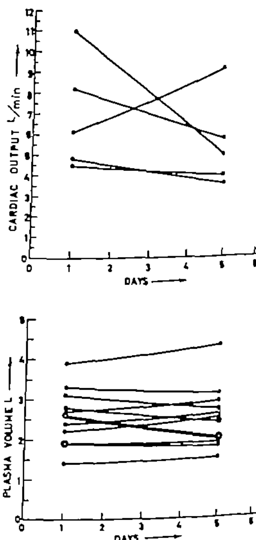


Fig 4 The variations in cardiac output and plasma volume occurring during the period under investigation.

cretion in the urine was slightly but not significantly reduced compared with that on the first control day

In addition there was found to be a slight but not significant increase in the serum urea from an average of 38 to 49 mg% in the 9 patients investigated

After the combined therapy there was an increase in the serum uric acid in 10 out of 11 patients, while it was unchanged in one. The average increase in serum uric acid 3 05 mg/100 ml, was significant ($P < 0.001$) (fig 2) The uric acid excretion over the three day period was reduced by an average of 48 mg/24 hours in comparison with the first control day This reduction was, however not significant

lowing a dose of 75 mg/day that more careful dosage will be necessary in long-term therapy

Summary

The purpose of this investigation was to see the degree to which hydrochlorothiazide accentuated the hypotensive effect of guanethidine.

The investigation was planned as a short-term one using an intravenous infusion of guanethidine given over 15 minutes. The dose given was 0 25 mg/kg body weight dissolved in 100 ml 5 glucose. This was given before and after 3 days oral treatment with hydrochlorothiazide, 75 mg daily

Intravenous infusion of guanethidine has marked hypotensive effect in hypertensive patients lasting from 6 to 8 hours in most cases. The effect increases in the sitting position and is greatest on standing. In some patients there was an initial increase in pressure lasting from 5—10 minutes.

After three days oral treatment with hydrochlorothiazide there is an increase in the effect of the same dose of guanethidine. In this period a marked increase in urinary output occurred in comparison with that in the previous control period. In addition an average weight reduction of 1 7 kg was found. All the patients showed a significant increase in Na and Cl excretion as well as in K excretion ($P < 0.001$) The serum Na concentration showed a slight, but insignificant, drop, while the K and Cl concentration were significantly reduced ($P < 0.001$) The alkali reserve and serum creatinine showed a significant increase, while the serum urea was slightly but insignificantly increased.

In 10 out of 11 patients the uric acid concentration in the serum increased, but none developed symptoms of uric acid arthritis.

The excretion of aldosterone in 24 hour urine specimens was examined in 8 patients before and after the combined therapy In one patient it was estimated for the following 9 days. Out of 25 specimens investigated the excretion was increased in 4 in 3 of which the increase occurred after treatment had ended

The cardiac output fell in 4 out of 5 cases after combined therapy

The orthostatic hypotension in 7 of the patients must be the result of an extra strong effect due to the administration of the guanethidine as an intravenous infusion. The electrolytic changes were probably due to the relatively intense hydrochlorothiazide medication.

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be regarded as considerably greater than that following oral medication The advantage of therapy combined with diuretics is that in addition to counter acting the water retention produced by the guanethidine, the diuretic potentiates its action.

In the three day period with hydrochlorothiazide medication (75 mg/day) there was a significant average reduction in the serum potassium to 3.5 mEq/l and in the serum chloride to 95 2 mEq/l while the alkali reserve increased to 30 5 mEq/l. These findings are indicative of electrolytic changes pointing to hypochloraemic alkalosis with a low serum potassium.

The creatinine excretion did not show any systematic change in the three day period when compared with that on the control day This indicates that the meas urements of the urinary output were satisfactory

The significant increase in the serum creatinine can possibly be explained on the basis of the pronounced hypotensive effect, however there was no significant increase in the serum urea

The cardiac output fell in 4 out of 5 patients. As mentioned before reduction in the cardiac output has been reported by others. The mechanism of this fall in cardiac output is stated to be dependent on the reduced filling pressure in the right atrium, as a result of pooling of the blood in the peripheral vessels together with the reduction in plasma volume and extra cellular fluid

In the majority of the patients there was an increase in the uric acid concentra tion in the serum after guanethidine hydrochlorothiazide therapy but none developed symptoms of uric acid arthritis. Increase in the serum uric acid during treatment with thiazide has been re ported by other authors (2 17 18, 32). The cause of the increased concentration may be reduction in the uric acid clear ance

Ayvazian and Ayvazian (2) state that a complex metabolic action is as likely as an effect via the renal tubules.

The catecholamine metabolite VMA was excreted in normal amounts during the investigation period in the one patient investigated The aldosterone ex cretion in the urine was more or less nor mal. This, however is influenced by so many factors, among others the electrolytes and the plasma volume, that the results are difficult to evaluate. Gifford et al. (13) found on the whole normal values for the aldosterone content in 24 hour urine specimens from hypertensive patients treated with thiazide diuretics.

No side effects, apart from orthostatic hypotension were recorded. This must however be considered to have been more marked than usual as the guanethidine was given as an intravenous infusion.

Conclusion

Intravenous infusion of guanethidine has a marked hypotensive effect in hyper tension. The effect increases in the sit ting position and is greatest on standing

After three days oral treatment with hydrochlorothiazide there is an increase in the effect of the same dose of guanethidine.

Combined therapy with these drugs should be suitable for long term treat ment in selected cases, but the intra venous administration of guanethidine is hardly suitable in view of the marked orthostatic effect.

In this short term investigation the electrolytic changes were so marked fol-

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Further Observations on Periodic Disorders

By

Erik Ask-Upmark

In 1938 I published a study on periodic fever (2) in 1945 another investigation on periodic diseases with special attention to the premenstrual condition in women (3) in 1945 a survey on periodic disorders in general (4) and in 1951 likewise a review of relevant phenomena (7) Other papers with bearing on this topic were published in 1950 (5) and in 1955 (8) Periodic occurrence of symptoms of various diseases had of course, been known for centuries (the attacks of malaria) and Hodgkin had called attention to the cyclic appearance of certain symptoms in the disorder that rightly carries his name. It was Murchison in 1870 who described the periodic fever that may occur in Hodgkin's disease, an observation which in the literature has been unjustly ascribed to Pel and to Ebstein René Mach, of Switzerland in 1940 described the intermenstrual syndrome (28) Reimann of Philadelphia, in 1948, assembled a series of cyclic disorders under the name of periodic disease and has in subsequent papers furnished valuable contributions to our knowledge (33, 34 35, 36 37 a, 37 b) The present paper aims to report some hitherto unpublished observations, to suggest a hitherto unknown treatment for intermittent hydrops of the joints, and to give a brief survey of our present knowledge of periodic disorders.[1]

Material

The material here to be described represents only a limited selection of cases rather than a systematic review of our material assembled during more than 20 years.

Case 1 Physician, aged 56. Since many years attacks of auricular fibrillation which start at 10-day intervals and the duration of which may be less than 1 day but also as much as 6 days. Six months ago he was presumed to have thyrotoxicosis, and conservative treatment with thiouracil preparations was started in another hospital. It had, however no effect on the occurrence of the cardiac attacks, nor were other preparations such as digitalis or chinidin of any use. He has observed, however that an attack may be stopped immediately if he gets angry

Case 2. Head secretary born in 1903, observed by myself repeatedly during the years 1949 and 1951—55 During this period and

Submitted for publication July 17 1962.

[1] The essential features of the paper were reported to the Swedish Society of Internal Medicine at its annual meeting in Upsala, Sept. 1958.

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was Scheuermann-kyphosis which might have taxed his pulmonary circulation. H had tried various remedies, also large doses of chinidin (3 g daily) but whatever he did the attacks appeared t their regular intervals. As always in paroxysmal auricular fibrillation he was distressed considerably by the alterations of the cardiac rhythm. He had gall stones as well and I tried in vain to persuade him to have a cholecystectomy. Years later I have met this man he has now permanent fibrillation and manages to carry on, being less disturbed by his present cardiac condition than by the attacks. H told me about the attacks that when some days had elapsed since an attack he somehow felt that the appearance of new attack was imminent and could not be prevented whatever he did He could prophesy for instance that an t tack was due next Sunday It is as if some substance is being accumulating in my body and when it reaches certain level the trigger is pulled.

Case 3. Farmer aged 75 Previously healthy Last 5 years increased frequency of micturition by night. Nov 1945 pains in left knee and ankle region, which eventually subsided. Dec 1945 pains in both legs particularly in the thighs. Jan. 1946 pains also in the lumbosacral region Since Dec. 1945 he has felt weak and his weigh has reduced About New Years Eve he had an attack of fever for few days, reaching 38 5 C similar attack had been present 8 days earlier He entered the clinic on Jan 7th and was found to be pale, weight obviously reduced, prostate had an uneven surface and was obviously of hard consistency Roentgen examination of the pelvis failed to reveal anything abnormal. He went home Jan 16th Abou week after this he got fever of about 39 5 for few days and the same was the case one week later Some sulpha-preparation was tried but he reacted unfavorably getting exanthema on his hands. Since Feb. 9th increasing temperature, being 39 C on the night preceding readmission Feb. 11th. There was now anemia (hemoglobin 68 %, red count 3 32 mill., white count 7,600, differential count normal) The level of acid phosphatase was considerably increased (3.3—6 IU) and when, in April, reexamination of the pelvis was undertaken the presence in the bone system of an osteoplastic carcinoma was evident His temperature whilst in the clinic is reproduced here (fig 1) It will be seen that there is a marked periodic fever until the introduction of stilbol May 5th (2 mg × 3) when it subsided. This case was observed 6 months before my own arrival in Upsala and referred to by my predecessor in his last lecture.

Case 4 Electrician, aged 31 This man had Hodgkin's disease and was repeatedly observed in our clinic during the course of almost one year when he died. A striking feature was the presence of periodic fever (Murchison) and the temporary enlargement of the spleen with each period of fever At the necropsy it was found that the spleen itself, although enlarged, did not present any lymphogranulomatous tissue at all. The enlargement was due to compression of the splenic vein by means of a large aggregate of retroperitoneal lymph nodes, severely infiltrated by the pathological tissue. It was obviously the rise and fall in size of these lymph nodes which had determined the variations in size of the spleen.

Case 5. Lawyer born 1919 Observed by us in 1948. Died in 1951 in another hospital in amyloidosis of spleen, liver suprarenals and kidneys (uremia)

His mother had, when 20—30 years old suffered from hydrops intermittens in the knee-joints. In later years she had such t tacks only rarely and she was entirely free from ttacks during her four pregnancies. One sister of his mother had also had when young, ttacks of hydrops intermittens in her knee-joints. She had since remained well for several years recently however the cycles had recommenced after an injury to one of the knees.

H had himself had motor accident in 1931 with injury of his head and his right side, particularly the hip-joint region. Roentgenological examination of the hip-joint was negative. In 1936 whilst playing football, he got kick against his right knee. The knee reacted with an exudation which disappeared in one week From this time, however he got an intermittent hydrops in his right knee, with intervals of 12—13 days. In 1942 a surgical exploration was made with an excision from the plicae alares the synovia was seen to be

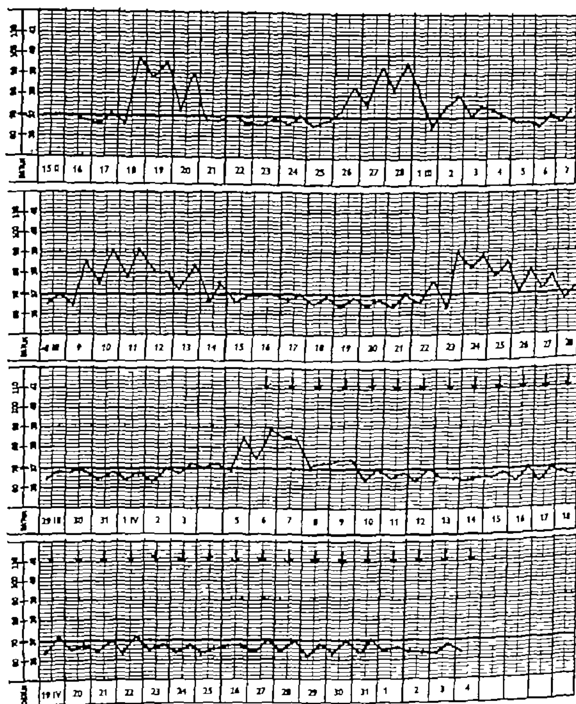


Fig. 1. Temperature chart of case 3. The periods of pyrexia could really be traced back to the end of Dec. 1945 with the same regularity. The curve here reproduced accordingly represents only a fraction of the course. The arrows indicate the administration of stilbol, by means of which the periods of pyrexia were abolished.

even years before he suffered from periodic attacks of auricular fibrillation. There was as a rule 6 days between the onset of the attacks, occasionally 4—5 days. The duration of the attacks was variable, from one or two hours up to 39 hours. The man was heavy (height 190 cm, weight 97 kg), he had some degree of arterial hypertension (200/110) and there

t first the diagnosis ankylosing spondylitis was suggestive, repeated X-ray examinations failed to show any progress of the affection. It was rather felt by the radiologists that Bechterew was not present (some degree of spondylolisthesis between L V and S I was present)

Case 7 Woman, aged 21 wife of an electrician. As child morbilli, pertussis, parotitis and on three (1) occasions varicellae. 1957 normal delivery after which, however she is supposed to have had an oophoritis. June 19th 1960 swelling and some pains in left knee-joint. After 10—12 days the swelling disappeared but later is returned again. In the surgical outpatient department her left knee was immobilized for fortnight by plaster of Paris but no reduction of the swelling was achieved. During this time the right knee joined the left with rapidly appearing and subsequently disappearing periods of hydrops. The periods have been approximately 10—12 days, as a rule only the left knee has been affected, once in while both of them and occasionally only the right one. She was admitted to our department Oct. 6th and kept there until Dec. 15th under the diagnosis hydrops articularis intermittens. On admission her general condition was good, temperature normal, pulse rate irregularly increased (80—100) and sedimentation rate 18/1 hour A swelling of her left knee was evident, whereas the ppearance of the right one was normal. Antistreptolysin titer 100, antistaphylococcal titer 1 4 sheep red-cell-agglutination test normal. Electrophoresis almost normal, no LE-cells to be found. On three occasions (Oct 18th, Oct. 29th and Nov 7th) the knee joint was punctured. The amounts of viscous liquid obtained were 23, 12 and 4 ml, of which 10 10 and 4 ml respectively were injected intramuscularly She was very much improved although some minute swelling still remained. During the last month in the clinic she also got small dose of dehison (5 mg × 2) which, however was discontinued in Feb. 1961 Feb. 21st 1961 she was again admitted to the clinic, where she was observed until March 9th. She was ery much improved, yet some slight swelling of both knee-joints was still evident, although much less than prior to her treatment. Being young woman with small child at home, she could not be retained as long as originally desired.

Case 8. This case has been described in an earlier publication of mine (5) A woman, aged 50, applied for medical assistance be cause of headaches since 10 years. A moderat hypertension and a slight anemia were manifest. For at least 5 or 6 years she had observed that her left foot and calf presented a considerable edema in connection with the menstruations. For the last 2 years, however she has had no more menstruations and yet the edema has continued to appear regularly every fourth week, lasting for few days at a time The patient was seen by us repeatedly large pitting edemas being encountered in her left foot and the lower part of her left calf and ankle. This edema was observable for 3 —4 days at regular intervals of 4 weeks.

Case 9 Female aged 31 in 1939 when I saw her for the first time. Employed as secretary by an editor Menarche when 13 periods regular until 1930 when they ceased for one year and a half. When they returned she got a ravenous hunger particularly during the week preceding the menstruation but to some degree also in between. The hunger was particularly for sweets she could eat up to 1,000 g sugar at a time. Since 1936 when the hunger was most pronounced, her parotid glands became swollen and board-hard, but when she satisfied her ppetite the parotids returned to normal size. When hungry she was warm, sweating and thirsty In between she felt cold and was not thirsty al all. Her weight has during these 8 years varied between 56 kg in 1930 and 100 kg in 1935 In connection with the hunger periods she was very sleepy when not hungry she was not sleepy at all. The hunger periods were by no means regular except for the premenstrual exacerbations. Her blood sugar had been examined repeatedly and was always found to be normal. She often had dreams, imagining that other people were in her bedroom, which they were not. For some time she had a feeling of weakness in her legs, particularly in the mornings, whereas they always improved in the evening She had been observed in various hospitals by numerous physicians, obstetricians and psychiatrists, and had been subjected to different kinds of treatment (such

red with hypertrophied cells. Since then his attacks have switched to the left knee. In 1944 he was observed for microscopical hematuria which was thought to be iatrogenic (arsenic) He then remarked that if he tried to avoid sodium in his food the hydrops was less pronounced. Also, when the hydrops was present he felt a dryness in his mouth. In 1946 his right hip-joint was operated upon and a free body in it was removed. In 1947 about Christmas the attacks became more pronounced the swelling increased for 3 days, remained at its maximum for 1 day and disappeared in 2 days more, the interval between the onset of each attack being 13 days. His antistreptolysin titer was now found to be more than 1,500 and one joint of his third finger was involved as well. Progesterone was without effect.

This case of hydrops articularis intermittens is instructive in several regards the occurrence of the disorder in the family its appearance in the patient after an injury to the knee and the eventual propagation to other joints with death in uremia from amyloidosis as the ultimate result.

Case 6 Farmer born 1927 Previously healthy In 1953 some attacks of syncope when standing erect for a while, for instance whilst attending funerals (he is tall and slender) In 1954 when aged 27 fracture of right femur and pulmonary embolism. 1955 he fell 5 meters from a roof and got a fissure of the left patella with hemarthrosis, for which he was immobilized in plaster of Paris for some weeks. When the plaster of Paris was removed, in August, there was still a swelling of his knee. In December increased swelling of the joint after working in deep snow This swelling started to disappear and to return with a regular itinerary of its own, 10 1/2 days elapsing between the onset of each swelling He was treated in other hospitals by various means intraarticular injections of hydrocortisone were of no avail, he then got local roentgen-treatment during two months without any effect whatsoever intraarticular osmium injections were tried in vain and the only relief he got was during 6 weeks in 1955 after a surgical removal of part of the synovia of the left knee. In the summer of 1956 the right knee imitated the left, the periods of 10 1/2 days still being maintained although each knee joint had its private time-table. It had been maintained that he was suffering from an ankylosing spondylitis as well. He entered our department in 1957 Nov 25th. He was a tall and slender young man, his sedimentation rate was 23 in 1 hour a systolic murmur was to be heard at the base of his heart. His back was very straight, without the usual lumbar lordosis. The left knee did present some thickening of the capsule and a slight hydrops. In the subsequent course there was a regular intermittent hydrops with an interval of 10 1/2 days between the onset of the swelling and with the right knee 5—6 days ahead of the left one The antistreptolysin titer was 1 000 units, the agglutination of sheep-erythrocytes negative Electrophoresis of serum proteins within normal limits. It was evident from the poor therapeutic results so far obtained that something else had to be tried. I decided upon the following treatment. The left knee was punctured and some 10 ml yellow viscous liquid withdrawn. This liquid was immediately injected intramuscularly This treatment was started Dec. 5th and when he left our clinic Jan. 31st he had got seven such injections. The sedimentation rate dropped from 23 to 6. During the treatment all the swelling disappeared at first in the right knee-joint, later also in the left. During the last 3 weeks in the clinic, when the sedimentation rate already had dropped to normal level and no symptoms from the knees were present, he got also a moderate dose of prednisone

This man has since been repeatedly observed in my office as well as in the clinic where he was observed for brief periods in 1958, 1959 and 1960. I have seen him personally last time Feb. 28th, 1961 There has been the striking feature that all swelling of the knees has entirely disappeared, except for a few weeks in the autumn of 1958, when he had a distortion of the left knee-joint after one puncture of the joint the swelling rapidly subsided. His antistreptolysin titer has been normalized and he is able to walk about and carry out all the various jobs connected with farming As for the supposed Bechterew disease X-ray examination of the sacroiliac regions in 1958 revealed some small usures on the iliac side where there also was some degree of sclerosis. The joint space itself, however was unaffected and although

mum in August, the palm of generalized bone diseases with their zenith in the late winter and early spring and their amelioration during the summer the predilection for the spring of tetany of asthma and of conjunctivitis aestivalis, the peculiar maximum of the suicides in May are all phenomena familiar to the clinician.

2. *The month.* In females the hormonal tides connected with ovulation and menstruation are reflected in the behavior of numerous disorders.

a) The premenstrual mental irritability is well-known phenomenon. For relief, the women may turn to overeating to cigarettes or even to alcohol — one patient of mine used alcohol regularly during the week preceding menstruation, otherwise she was a teetotaller In reviewing our material of intoxications with barbiturates and suchlike our young colleagues, Bernadotte af Wisborg and Schuback (10) tried to find out whether the suicides and the attempted suicides presented any correlation to the menstrual cycle, the inference being that an increased frequency would be expected during the premenstrual period. Owing to deficient information in the records they were not able to pass any judgment on this point Some years later British authors had better luck and were able to point out the lethal hazards of the luteal phase of the menstrual cycle, such as suicides and accidents (29 30)

b) Several disorders of the nervous system likewise present an exacerbation in the premenstrual phase, an amelioration after the onset of the menstruation. Such is the case with many instances of migraine, with myasthenia gravis, with multiple sclerosis, with meningeomas, with arteriovenous aneurysms in the head with subarachnoid hemorrhages, with the peculiar cerebral syndrome of Takayashu's pulseless disease" with carotid sinus syncope.

c) Numerous skin disorders such as seborrhoea, psoriasis and herpes simplex are apt to present an exacerbation in the premenstrual phase. The same may be the case with various allergic affections of the skin as well as with bronchial asthma and notably also rheumatoid arthritis, conditions incidentally which almost always tend to improve during pregnancy The mother of our case no. 5 seems to be instructive in this regard her hydrops articularis intermittens was absent during her four pregnancies.

d) There is usually a retention of fluid during the premenstrual phase, as easily discerned from the impressions on the skin of the face made by the laces of the pillow This observation tallies well with the decreased volume of urine produced prior to menstruation and the increased flow after its onset. Occasionally this retention may lead to monthly attacks of pulmonary edema, particularly if there is a mitral stenosis about I have had the opportunity to witness this sequence of events in some instructive instances. A more atypical type of edema, to be compared with the Quincke-type is represented by case 8 described above. Paroxysmal tachycardia, being an equivalent of migraine, has the same predilection for the premenstrual phase of the cycle as has migraine itself. Among other similar equivalents of migraine may be mentioned the so-called acute abdominal distention ("bloating syndrome" of the Americans) In 1945 I described a young girl whose waist during the premenstrual phase increased from 65 cm to 96 cm giving her each month the appearance of advanced pregnancy this enlargement of the abdomen was to

as prolan injections etc.) but all without avail. She felt best if she could get angry: "the anger is carrying me along".

This young woman was observed repeatedly in our department. The only positive observation was a slight pleocytosis in the cerebrospinal fluid obtained by suboccipital puncture. It was, therefore, concluded that some kind of encephalitis might have been present. She improved considerably on amphetamin. She later married and had children, and in spite of a certain nervous instability she is able to cope with her family duties and her work as a secretary.

Case 10. Woman aged 22. Parents alive and healthy. As a child scarlet fever and diphteria. Constipation as long as she can remember necessitating various remedies such as castor oil etc. Menarche when 13—14 years of age. Works with her father who is an innkeeper. Three years ago cessation of the menstruations until 6 months ago, when they reappeared, apparently after a car accident when she hurt her head and her teeth against the steering wheel. Two and a half years ago nervousness for no apparent reason, occasionally also diplopia which since has occurred repeatedly. Two years ago the actual history started. The main complaint is the periodic appearance of a ravenous appetite when she wolfs down everything edible, to which she had easy access in the inn. She indulged particularly in sweet and fat food, she might for instance easily consume 1/2 kg butter and a big loaf of bread a day and she was particularly anxious to get fish as well as eggs in ample amount: meat did not suit her in the same way. Such periods of hunger initially had a duration of about 2 months, with an equally long interval between them, but eventually both the hunger periods and the intervals have diminished to about 4 weeks each. During the periods of ravenous appetite she was also very thirsty consuming at least two and frequently three liters of water a day. She was warm and sweating and she had tachycardia. She also felt a bit drowsy although no real somnolence was present: on the contrary she had to get up by night to help herself to more food. During the intervals she hardly touched any food, she felt no thirst, yet the amount of urine produced during 24 hours was large (it was reduced during the hunger periods): she felt cold and she had no tachycardia. Her weight oscillated about 16—18 kg between its maximum at the end of the hunger period and its minimum at the end of the interval. She was seen by myself repeatedly. During the hunger periods her skin was warm and moist and flushed: she had tachycardia and the pulsations of the femoral artery were audible. During the intervals her skin was cold and dry: she had no tachycardia and no pulsations were to be listened in from the femoral artery. She gave the paradoxical impression of a thyrotoxicosis when increasing in weight, of a myxedema when decreasing in weight. Her blood morphology was normal, as was her gastric secretion (free hydrochloric acid present). When first seen the Hoffman sign was positive on both hands, her olfactory sense was absent on the right side: neurological examination otherwise negative. Since her car accident 6 months ago, she has suffered from considerable headaches behind the eyebrows.

This case had for two years had a periodic appearance of morbid hunger appearing several days a month but without any relation to the periods. She was analyzed in our clinic and it was felt that there might have been an encephalitis, although conclusive evidence was difficult to obtain. The case was observed in 1939—40 when we had no facilities for electroencephalography nor for angiography.

Comment and discussion

Cyclic periodicity so characteristic of numerous physiological phenomena — pulse, temperature, respiration, blood pressure, liver activities and so forth — is repeatedly encountered in the clinic. If the discussion be confined to rhythms of one year and less the following examples may be given.

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r In glomerulonephritis the renal reaction is apt to occur 7—10 days after the angina tonsillaris, and the same holds for the joint affliction in rheumatic fever although the interval here is slightly longer In the infectious diseases the period of incubation may vary between less than 3 days, as in scarlet fever to 3 months or more in inoculation hepatitis. However if all periods of incubation are plotted in a diagram the maximal density of the points will be found around 7—10 days. The production of antibodies and the reaction between antigen and antibody obviously has a rhythm of its own. In periodic fever caused by malignant tumors, such as the Ewing sarcomas or the carcinoma osnum in prostatic carcinomas, the periods of fever will usually amount to 5—10 days with an interval of corresponding duration. The Murchison fever in Hodgkin's disease has a similar rhythm although the cycle is usually somewhat longer (about 3 weeks between the onset of one period of fever to the onset of the next bout of pyrexia) The regular periodicity of hydrops articularis intermittens has, as a rule, 10—11 days as its cycle. In a most instructive case of Cushing's disease in a woman, previously deprived of her ovaries, Birke and collaborators found regular periodicity in the excretion of 17-ketogenic steroids in the urine with a maximum to be noted about every 10th—12th day(12)

4 *The day* The well-known physiological variations during the 24-hour cycle are t be observed in most biological functions such as temperature, heart rate and blood pressure, sleep respiration and various glandular activities. The investigations of Forsgren on the hythmic function of the liver may be quoted as an example by night the assimilatory functions prevail, by day the dissimilatory (bile-production) (42) Le courage à deux heures du matin" was rightly identified by Napoleon and the predilection of suicides for the early morning hours is familiar to every physician. The vulture picking on the liver of Prometeus every night is perhaps the earliest reference to a 24-hour rhythm in connection with human disease if the pains in the bones of Job by night are excepted The predilection for the night is particularly outstanding in the various diseases characterized by paroxysmal attacks. Gout, epilepsy asthma, pulmonary edema, pains in the bones in generalized disorders of the bone system are some of several examples.

Although no attempt will be made to explain the periodicity of all the disorders here exemplified, certain points may be mentioned with a bearing on conditions such as hydrops articularis intermittens, paroxysmal auricular fibrillation and periodic fever in certain tumors of the bone system (Ewing sarcomas, carcinomas)

1 The condition may be due to some auto-immunity reaction, being self perpetuated in much the same way as a chronic glomerulonephritis, an acquired hemolytic anemia or a Hashimoto goiter

Our attempt, so far successful, to break the vicious circle in the hydrops articularis intermittens, by injecting the intraarticular fluid intramuscularly perhaps lends some support to this theory Another suggestive piece of evidence may be the frequent occurrence of a traumatic injury to the joint, initiating the history of a hydrops articularis intermittens this injury may play the same rol in such a case as the surgical intervention so frequently heralding the ppearance of Hashimoto. In this connection the reac

be elicited by a meal (3) An explanation of this phenomenon has been given in another publication of mine (with Frantzell) (6) Arterial hypertension and its coronary sequences may present exacerbations in the premenstrual phase, as may also the appearance of extrasystolic beats Acrocyanosis as well as doigts morts are always worst prior to menstruation and tend to improve after its onset

e) The well known alterations of the body temperature during the menstrual cycle may be recalled In Addison's disease an exacerbation of the symptoms is likely to occur prior to the menstruation and similarly in the so-called menstruation tetany which rather should be termed premenstrual tetany The behaviour of the eosinophil leucocytes during the monthly cycle has been analyzed by Davis and Hulit (19) There is some evidence that the excretion of citric acid may vary during the monthly cycle and that these variations are of importance for the appearance of recurrent concrements in the urinary tract (45)

f) From the digestive tract the most striking monthly periodicity seems to be constipation in young women which almost always is more pronounced in the premenstrual week. For a more detailed description of various other monthly periodicities of the digestive tract reference may be made to an earlier publication (3) and to Mach (28) who particularly calls attention to the intermenstrual phenomena connected with the ovulation.

g) The skin pigmentation has close correlations to the phases of the menstrual cycle in that there is always a darkening of the skin in the premenstrual week (31) I have also had the opportunity of observing young girls who with astonishing regularity got one pigmental mole with each menstruation during the premenstrual week there was a local itching which had to be scratched. The mole appeared reddish, in the scratched region immediately before the onset of the menstruation and darkened rapidly (9a)

h) Cyclic sciatica, due to endometriosis affecting the sciatic nerve and closely related to the menstrual periods have recently been described by Head and collaborators from the Mayo Clinic (22).

3 *The week.* External conditions pertaining to the week underlie the accumulation of motor accidents to the week end the monday head experienced by workers in factories producing nitroglycerine, the brass-malaria or zinc fever which formerly was a common disease in Upsala (fever starting with its peak monday afternoon and eventually subsiding during the week) and the respiratory reactions sometime described as occurring mondays in byssinosis.

There is, however also a "biological week which is not related to the calendary In days gone by before the introduction of chemotherapy and antibiotics, the spontaneous crisis in lobar pneumonia occurred on the 7th or the 9th day Drug fever usually appears after 7—10 days and the same interval is to be noted in serum sickness. In pernicious anemia the increase of the reticulocytes in response to treatment tends to occur after 6—7 days. Bee-stings may unexpectedly show a severe reaction 7—8 days after the accident. If a person with gout is operated upon for some disorder (for instance by means of a cholecystectomy) an attack of gout is apt to appear about 5—7 days after the surgical intervention similar attacks of gout may occur with the same interval after a coronary infarction (9b)

3 A brief review is given of various cyclic disorders in clinical medicine, and attempts to explain some of them are briefly outlined.

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tion of patients with gout may be referred to — surgical interventions or pathological necroses such as cardiac infarctions, may initiate an attack — and a similar explanation has also in previous papers of mine, been given for the appearance of Duplay's syndrome in connection with necrosis of the tissue — as in cardiac infarctions but also in burns and in cerebral infarction.

2. The accumulation of a substance produced by the body or by the tissue involved so as to trigger a reaction when it has reached a certain level is another interpretation, which of course is entirely compatible with the one mentioned below (1) Already in 1942 Bramwell and King in their Principles and Practice of Cardiology have referred to the repeated attacks of auricular fibrillation as follows "The way in which these paroxysms recur at more or less regular intervals, even in otherwise healthy subjects, is very suggestive of some biochemical cumulative process which automatically explodes when it reaches a certain stage of its development, and then starts to re-accumulate, preparatory to another paroxysm. As for the nature of this substance it may be some antibody or other compound perhaps hormonal in character (the etiocholanolone fever of Bondy) (13 25) The monthly periodicity connected with the menstruations is at least suggestive in this regard and the periodic excessive production of hydrocortisone described by Hökfelt et al. (24) in a case of pituitary tumor as well as the periodic variations in the excretion of 17 HO-ketosteroids reported by Birke et al (12) represent additional evidence.

3 A central regulation of rhythmic phenomena is very tempting to assume However this does not necessarily concern the centrencephalic structures but perhaps hormonal impulses (as exemplified by the premenstrual disorders) or a rhythm of the reticuloendothelial system of its own, a rhythm where perhaps the antibody production and the antigen-antibody response may be involved, and which may have correlations to the endocrine system as well. The well-known influence of cortisone on the antibody antigen response and the periodicity occasionally to be noted in hydrocortisone production (23) may fit into the pattern of reticuloendothelial response. The same may hold for the peculiar lymphocytopenia in the adrenocortical syndrome of Cushing the importance of the lymphocytes and their reticular mother-cells for antibody formation has recently been discussed in an editorial in J Amer Med. Ass. (*180* 1052 1962)

Summary and conclusions

1 The present paper describes a series of periodic phenomena observed in our clinic

Auricular fibrillation (case 1 case 2)

Hydrops articularis intermittens (case 5 case 6 case 7)

Periodic fever in carcinosis (case 3)

Murchison fever with periodic enlargement of lymph nodes in Hodgkin's disease (case 4)

Premenstrual edema of the foot, occurring regularly each month even after the menopause (case 8)

Periodic hunger with enlarged parotid glands (case 9)

Periodic hunger drowsiness and other phenomena (case 10)

2 Attention is called to a new method by means of which hydrops articularis intermittens may be relieved intramuscular injections of the intraarticular fluid of the patient.

5. A brief review is given of various cyclic disorders in clinical medicine, and attempts to explain some of them are briefly outlined.

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Long term Treatment with Corticosteroids in Rheumatoid Arthritis

(Over a Period of 9 to 12 Years)

By

J. Boye Nielsen, Aa. Drivsholm, F. Fischer and K. Brochner Mortensen

The demonstration in 1949 that corticotropin or cortisone therapy brought about an immediate improvement in the condition of many patients suffering from rheumatoid arthritis gave rise to widespread optimism. Expectation turned to deep scepticism when increasing experience showed that in many cases the improvement obtained was merely of a transient nature, or could be maintained only by such high doses that continued treatment involved a considerable risk of serious side effects. There has been — and still is — much discussion, therefore, on the indications for the use of corticosteroids as long-term treatment in patients with rheumatoid arthritis.

There is no doubt that many still employ the treatment on too wide indications, some have quite given it up, while others continue to use it on very narrow indications.

The literature on the subject is overwhelming, but nevertheless only few studies have been published in which the patients have been followed closely throughout their treatment over a long period of time (1, 2, 3, 4).

Material

The long-term treatment of a group of patients suffering from rheumatoid arthritis was started in Medical Department A, University Hospital, in August 1950, and these patients have been followed closely since then.

The results of current observations have been published on a number of occasions during this period. A general account of the first few years' investigations was given in 1955 by Fischer (5).

The patient material comprises 15 men and 35 women, the majority of whom were in the age group 30—50 years at the start of the steroid treatment. All patients had active progressive rheumatoid arthritis, the duration of disease varying from 3 months to 22 years (mean 6 years). Previously they had been treated intensively with conventional methods for example, most of them had received quite large doses of sanocrysin.

ACTH was used at first, cortisone shortly afterwards, and from 1955 onwards prednisone.

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Table I 50 patients with rheumatoid arthritis treated with steroids

Group		
A	Treatment discontinued (unsatisfactory response)	9
B	Treatment discontinued (complications)	16
C	Treatment discontinued (remission)	9
D	Treatment continued	16

No other steroids have been used in this material.

In addition to the steroid treatment, physiotherapy was given. The use of analgesics was reduced as far as possible. Five patients underwent orthopaedic operations in the course of the period under consideration. Otherwise there has been no other treatment, apart from that necessary in manifest complications.

The patients have been followed closely The main rule — only rarely broken — has been for the patients to have an out-patient examination at least once a month. The control examination has been done by the same doctor specially chosen for the purpose, and serving over a period of several years at a time.

Particular emphasis was given to an examination of the general condition, both physical and mental, as well as the state of the joints. At the start, the following values were determined at each examination but later on they were determined a number of times annually weight, blood pressure, Hb and ESR, together with urinalysis for protein and sugar

An X ray examination of stomach heart, lungs and lumbar column was mad at least once a year

Patients and their relatives received explicit instructions to report immediately to the department on the slightest sign of complications or changes n the patient s usual condition.

Classification

Group A

Table I shows that steroid treatment was discontinued in 9 patients, as sufficient effect was not obtained at a reasonable level of dosage They were almost all in a rather late phase of the disease and characterized by irreversible changes in the joints.

Table II Treatment discontinued because of complications or death

Sex	Age when treatment was stopped	Duration of treatment (mths)	Reason for discontinuing treatment
♀	36	1.5	Mental disturbances
♀	29	2.5	
♀	50	12	
♀	35	43	
♀	54	90	
♀	53	12	Gastric ulcer
♂	47	12	
♂	47	1.5	Death from pneumonia
♂	39	19	
♂	50	84	
♀	60	89	
♂	58	111	
♂	39	113	Death from pulmonary complications with respiratory insufficiency after operation for gastric ulcer.
♀	51	73	Death from? cerebrovascular accident.
♀	54	96	Death from cardiac insufficiency
♂	71	115	Death from coronary occlusion.

Group B

The treatment was discontinued in 16 patients because of complications or death.

In five of the 16 patients, the treatment was discontinued on account of mental disturbances, mainly in the form of depression or pronounced restlessness. The mental disturbances disappeared in all cases on withdrawal of the treatment.

In two patients the treatment was withdrawn because of gastric ulcer

The other 9 patients died under the following circumstances

Shortly after starting the treatment two patients died from pneumonia whilst being treated as an outpatient. In spite of the instructions provided, neither the patients nor their relatives had notified the department. Neither of these patients had received an extra supplement of cortisone.

A 58-year-old man, who had received steroid treatment for a period of 9 years, also died whilst receiving out-patient treatment, presumably as a result of an untreated pneumonia.

A 50-year-old man who had been under steroid treatment for 7 years was admitted to the department in a state of shock, with loss of consciousness and suffering from pneumonia. The patient could not be brought out of shock in spite of intensive therapy with intravenous hydrocortisone and antibiotics, and died a few hours after admission. Autopsy showed pneumonia of the right lung and hypoplasia of the suprarenal glands.

A 60-year-old woman, under steroid treatment for 8 years, was admitted with staphylococcal pneumonia. This was complicated by empyema and pyopneumothorax, and terminated in death in spite of intensive medical and surgical treatment. At no time did the patient show any signs of shock.

A 59-year-old man, under steroid treatment for 9 years and with full working capacity all this time, developed a large gastric ulcer. The ulcer perforated during his admission to the surgical department, for which suture was carried out, together with vagotomy. Wound healing was retarded, and the wound had to be resutured several times. Six months later there was haematemesis and melaena, which necessitated local resection of the gastric ulcer and shortly afterwards gastric resection was performed on account of increasing haemorrhage. Ten days after the last intervention the patient died in a state of high fever with respiratory insufficiency but with no sign of shock. Autopsy was refused.

A 51-year-old woman had received steroid treatment for 6 years with good effect, and had full working capacity. She had suffered from migraine since childhood. One hour before death she was quite well, but then suddenly complained of headache, went to bed, and was found dead shortly after. Autopsy was not performed, but the cause of death had presumably been a cerebrovascular accident, even though no arterial hypertension or signs of heart disease had been demonstrated previously.

A 54-year-old woman who had received steroid treatment for 8 years, and who had had atrial fibrillation for two years, died in increasing cardiac insufficiency without any previous sign of arterial hypertension.

A 71-year-old man had received steroid treatment for 9 1/2 years and his working capacity and general condition were good. Without prior dyspepsia, he was admitted with severe haematemesis (gastric ulcer) necessitating gastric resection. After various complications the patient started to improve, but then died suddenly from coronary occlusion.

Group C

In 9 patients, the steroid treatment was withdrawn following gradual reduction of the dose because of complete or almost complete remission. On follow-up (the last occasion being May 1962) 8 of these patients had shown no signs or symptoms of recurrence of their joint disease at any time after withdrawal of the steroid treatment. The last patient in this group showed signs of renewed activity 1 1/2 years after withdrawal of steroid treatment. Sanocrysin treatment was given with some effect. Three years later the patient again had symptoms from the joints, and during the following four years was given 10 mg prednisone daily by the family physician. At an out-patient examination in the department in May 1962 6 months after renewed withdrawal of the steroid treatment, no signs of active rheumatoid arthritis could be found, but on the other hand there were clear signs of osteoarthritis.

Group D

This comprised 16 patients who continue to receive steroid treatment.

Duration of therapy

Table III shows the duration of therapy in the various groups.

Interruption of the treatment because of insufficient effect (group A) occurred in particular during the first few years.

Table III Duration of therapy

Years	Total no. of patients	Group			
		A	B	C	D
0—1	10	5	2	3	—
1—2	9	3	5	1	—
2—3	2	1	—	1	—
3—4	3	—	1	2	—
4—5	0	—	—	—	—
5—6	0	—	—	—	—
6—7	2	—	1	1	—
7—8	4	—	3	1	—
8—9	1	—	1	—	—
9—10	10	—	3	—	7
10—11	8	—	—	—	8
11—12	1	—	—	—	1
Total	50	9	16	9	16

Withdrawal of the treatment because of complications (group B) took place in 7 patients during the first two years (two of these patients died) in one patient after four years and in 8 patients after 6 to 10 years treatment (7 of these patients died)

In 7 patients, there was remission in the course of the first four years, and in two patients, after 6—8 years (group C)

In the remaining 16 patients (group D) steroid treatment was given for 9—12 years.

The steroid dosage used was 5—15 mg (mean 11 7 mg) prednisone per day The daily consumption of analgesics was 0—6 tablets of codeimagnyl[1] (mean 2 6 tabl.)

Result of treatment (group D)

Various methods have been tried to evaluating the result of steroid treatment in the 16 patients who continue to receive this treatment. Assessment of work

1 tablet of codeimagnyl = codeine phosphate, 10 mg, acetylsalicylic acid, 500 mg, magnesium oxide, 70 mg.

Table IV Capacity for work (group D)

Class	Before treatment	During treatment
I Complete ability	0	2
II Adequate ability	1	10
III Limited ability	9	3
IV Incapacitated	6	1

Table V Side effects in 16 patients still on corticosteroid medication (May 1962)

(Group D)	No.
Moon-face	10
Weight gain (> 5 kg)	2
Mental disturbances	2
Hypertension	3
Cardiac enlargement	3
Oedema	3
Peptic ulcer	3
Dyspepsia	1
Ecchymoses	10
Increased halisteresis	10

ing capacity before and during treatment provides the best picture (table IV)

Nine to 12 years ago before starting the treatment, only one was capable of work, compared with 12 today Six were completely incapacitated then, compared with one now

Undesirable effects and complications

A number of the all too well known undesirable effects and complications have been observed in the patients throughout the years These effects were partly due to the induced hypercorticoidism, and partly to hypofunction of the adrenal cortex. Table V shows the side effects present in the patients who continue to receive steroid treatment.

I INDUCED HYPERCORTICOIDISM

In this group, a number of the undesirable side effects are hardly of great significance. They include for example signs such as "moon face" (observed periodically in all our patients) slight increases in weight and disturbances of menstruation. Changes in carbohydrate metabolism have not presented a problem in this material thus there has been no case of steroid-induced diabetes mellitus.

Mental disturbances These have been of greater significance, however and were observed in 12 female patients, constituting indications for terminating the steroid treatment in 5 of these cases. However only milder and transient mental disturbances have been found in the present material.

Arterial hypertension. Elevated blood pressure — with or without enlargement of the heart — has been observed in about 1/3 of our patients during the periods in which ACTH or cortisone has been given. After changing to prednisone treatment, this side effect has not been of any practical importance. As mentioned, two patients died from heart disease and one possibly from a cerebrovascular accident, but in all three cases the blood pressure had been normal throughout the entire period of treatment.

Gastric or duodenal ulcer Peptic ulcer developed in a total of 9 patients during the course of steroid treatment. In two of the patients the treatment was discontinued for this reason. In three patients who continue to receive steroid treatment, roentgenological signs of scars after supposed ulcer have been visible for several years. Two patients have continued treatment, after having undergone gastric resection. Finally two patients died during the post-operative phase following gastric resection, from coronary occlusion and pulmonary complications with respiratory insufficiency respectively. In three patients the ulcer was localized to the duodenum, in two to the prepyloric region, and in four it was in the stomach proper. In most of the patients the ulcers were asymptomatic, diagnosed by routine roentgenological examination. In two cases the gastric ulcer perforated, in two other cases there was severe haematemesis.

Infections. Pneumonia occurred in 7 patients, 5 of whom died. Three of these were not in hospital at the time of death, and they did not get the extra supplement of cortisone which is vital in such situations. Two patients died in the department, one of them in irreversible shock in spite of intensive therapy while the other died from empyema, pyopneumothorax and septicaemia. Other infections have not caused any major problems in the present material. Thus, upper respiratory infections have not been strikingly frequent. Pyuria has been observed on one occasion. One patient has had necrotizing renal papillitis and intermittent proteinuria, but normal serum creatinine. Apart from this, none of the patients under treatment have proteinuria or elevated serum creatinine.

Haemorrhagic diathesis. There have been minor subcutaneous haemorrhages at intervals in a total of 19 patients, 10 of whom are among those continuing the treatment. Detailed examination of the blood coagulation properties in these patients, however showed nothing abnormal. On the other hand, the capillary resistance was found to be reduced in most cases.

Vascular changes of the periarteritis type have not been found in the present material, and histological studies from the four autopsies performed have not

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Undesirable effects and complications

A number of the all too well known undesirable effects and complications have been observed in the patients throughout the years. These effects were partly due to the induced hypercorticoidism, and partly to hypofunction of the adrenal cortex. Table V shows the side effects present in the patients who continue to receive steroid treatment.

Summary

An account is given of the results of long-term treatment by means of adrenocorticosteroids in 50 patients with rheumatoid arthritis.

The treatment was withdrawn in 9 patients because of the unsatisfactory result, in 16 patients because of complications or death, and in 9 patients because of remission. In the remaining 16 patients, the steroid treatment was continued for 9—12 years. Before the treatment only one of these patients was able to work, in contrast to 12 patients now. 6 patients were completely incapacitated in contrast to one now.

The undesirable side-effects and complications experienced throughout the years are described. A total of 9 patients died, three of them in acute adrenocortical insufficiency.

Otherwise, the most important undesirable effects and complications have been mental disturbances (12 patients), gastric or duodenal ulcer (9 patients), decalcification of the bony skeleton (spontaneous fractures in three patients), pneumonia (7 patients) and acute adrenocortical insufficiency.

It is felt in conclusion that it is possible to carry out the long term treatment of patients with rheumatoid arthritis by means of adrenocorticosteroids, and obtain an improvement in their working capacity. The risk of complications is however so considerable that the treatment should be employed only on very narrow indications, and under particularly careful control.

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shown vascular changes, nor has amyloidosis been demonstrated

Bony changes In a total of one third of the patients, routine roentgenological examination showed increasing halisteresis of the spinal column. Fractures occurred in a total of 8 patients. In 5 cases, the fracture occurred following trauma which must be regarded as adequate, and in these patients bone healing progressed normally In three patients, however there was compression fracture of the lumbar spinal column which must be regarded as pathological. Osteoarthritis appears to occur with increasing frequency in the material, and localized to joints that seemingly have not been affected by rheumatoid arthritis. It is not possible to decide whether this is solely a consequence of the increased mobility due to the symptomatic, analgesic effect of the steroid or whether a more direct steroid effect is involved of a catabolic nature. Preventive treatment with anabolic steroid has not been included in the standard regime.

II HYPOFUNCTION OF THE ADRENAL CORTEX

Three of the patients in the present material possibly four died from pneumonia presumably as a result of adrenocortical insufficiency A further five patients developed adrenocortical insufficiency with shock, but recovered during rapidly instituted and intensive treatment with adrenocorticosteroids. Atrophy of the adrenal cortex was demonstrated in all four patients coming to autopsy

In an attempt to counteract development of hypofunction of the adrenal cortex, various workers have suggested intermittent treatment by ACTH concurrent with the long term treatment by adrenocorticosteroids. A stimulation treatment such as this has not been employed in the present material, on the grounds that such a nonphysiological, intermittent stimulation could involve a certain risk of adrenocortical apoplexy and because it would still not be possible to omit prophylactic cortisone supplement in a stress situation, as a properly functioning adrenal cortex cannot be expected in spite of intermittent stimulation.

Conclusion

The conclusion must be that steroid treatment of patients suffering from rheumatoid arthritis can be given over a long period and that some of these patients can be kept in a reasonably satisfactory condition, so that their working capacity can be retained. However with increasing duration of treatment, the risk of complications is considerable, and the recognition of these so difficult that the treatment should only be used on very strict and narrow indications.

The main rule is that as soon as the effect has become manifest the dosage should be reduced and an attempt made to terminate the steroid treatment as soon as possible Experience shows, however that this offers considerable difficulty No attempt should be made to achieve complete freedom from symptoms, and instead the residual symptoms should be counteracted by means of analgesics.

Long term treatment in the true sense should only be given on very narrow indications, namely in cases with marked activity rapid progression and where the disease cannot be brought under control in any other way In such strictly selected cases, steroid treatment can be of value and for the time being indispensable, but it is only justifiable when carried through under very close supervision.

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From the Departments of Pediatrics and Clinical Physiology and the Chest Clinic, the University Göteborg, Sweden

Spirometric Studies in Normal Subjects

I. Forced Expirograms in Subjects Between 7 and 70 Years of Age

By

E. Berglund, G. Birath, J. Bjure, G. Grimby, I. Kjellmer, L. Sandqvist and B. Söderholm

There is an increasing demand for simple screening tests for ventilatory performance. With the aid of such tests individuals may be selected for more detailed analysis of pulmonary function. The spirometric methods meet this need if the apparatus used does not distort the recordings and if a normal material is available from the population in question. A spirometer which meets the above mentioned demand has been described by Bernstein et al. in 1952 (1) but so far no normal material has been examined with this apparatus in the Scandinavian countries.

The present study is the result of the collaboration between three different clinics. Independently but with identical techniques, two studies on adult subjects and one on children have been performed, the subjects being taken from the city of Göteborg.

Material

The material consists of 296 males and 201 females aged 7 to 70 years. It is composed of three parts, each part studied at one separate clinic. Each subject is judged from history and examination to be physically healthy and normal. For further details see the separate reports where each part of this material is described (2, 3, 6).

Method

Apparatus

The spirometer used was a slightly modified version of the one described by Bernstein et al. (1). This type of spirometer differs from those previously used in the following respects: it has a very low flow resistance, minimal distortion of the recorded curves and a small recording error at respiratory rates up to 110 respirations per minute (RPM). These advantages have been achieved

1) by making the connecting tube wide (5 cm inner diameter) and supplying it with a rubber mask instead of the usual mouthpiece,

2) by reducing the mass of the moving parts,

3) by increasing the cross-sectional area of the bell, thus reducing the acceleration of the bell, the intraspirometric pressure changes and the oscillations in the water-jacket, and

4) by modifying the water-jacket since the outer annular surface area is larger than the inner one, the large mass of water outside the

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Table I. Regression equations for the ages 7–70

Males	$\log_e VC$	$= 2.512 + 0.4395 \log_e A - 0.01525\ A - 340.80/H$
Females	$\log_e VC$	$= 3.077 + 0.3567 \log_e A - 0.01472\ A - 366.40/H$
Males	$\log_e FEV_{1}$	$= 2.294 + 0.4116 \log_e A - 0.01731\ A - 286.13/H$
Females	$\log_e FEV_{1.0}$	$= 1.994 + 0.4738 \log_e A - 0.02262\ A - 293.31/H$

A = age in years H = height in metres.

Table II. Simplified regression equations for adults

	Sex	Regression coefficients		Constant	R. S. D.
		Age, yrs	Height, m		
VC (l)	♂	−0.020 ± 0.004	+4.81 ± 0.66	− 2.81 ± 1.20	0.50
	♀	−0.022 ± 0.004	+4.04 ± 0.35	− 2.35 ± 1.61	0.40
FEV (l)	♂	−0.033 ± 0.004	+3.44 ± 0.56	− 1.00 ± 1.22	0.50
	♀	−0.027 ± 0.004	+2.67 ± 0.84	− 0.54 ± 1.44	0.36
FEV ( )	♂	−0.373 ± 0.058	—	+91.79 ± 15.72	7.19
	♀	−0.261 ± 0.033	—	+92.11 ± 21.42	5.44

down and attain his normal respiratory level, and to reveal any leakage around the mask, in which case the resting respiratory level tended to rise or fall.

1) *Vital capacity* (*VC*)[1] The subject was instructed to inspire maximally and then expire as deeply, but not as fast as possible. Three such tests were performed.

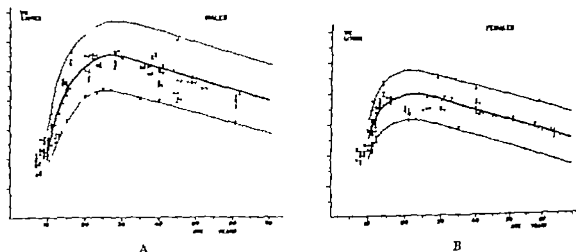
2) *Forced expiratory spirogram* (*FES*) To obtain an FES the subject inspired maximally and then expired as forcibly and completely as possible beginning the expiration abruptly. During the maximal inspiration the kymograph was switched over from the low to the high speed. The subjects were encouraged to increase their efforts for each test, and the tests were repeated until no further improvement could be observed. In general 3—5 FES were recorded. The following measurements were made: the forced vital capacity (FVC), the forced expiratory volume in 1 second ($FEV_{1.0}$) and the $FEV_{1.0}$ expressed as a percentage of VC (FEV)

The starting point of the expiration was determined as the point on the spirogram where the first deflection occurs, whether the curve immediately takes on its steepest slope or shows an initial part of flow acceleration. Since both types of expiratory flow have been recorded with a pneumotachograph (10, 13) the variation of the shape of the spirometer tracing is not an artefact.

As we are dealing with maximal function, mean values were not calculated. Each case is represented by its largest VC (or FVC) and largest $FEV_{1.0}$, whether these both values were found on the same curve or not.

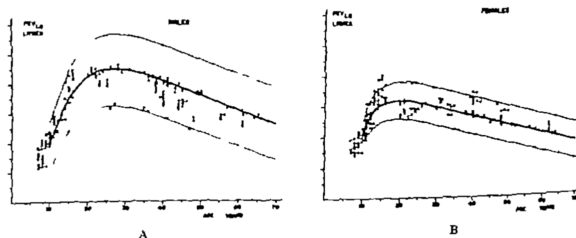
All volumes are given at ambient temperature and pressure saturated (*ATPS*) for the following reasons. The procedure of calculation is simplified, since different correction factors would have to be used for static and dynamic lung volumes: the temperature rises in the spirometer and equilibrium is probably not reached during the dynamic tests (cf. Ingelstedt (7)). The error introduced by omitting correction in repeated investigations is small in comparison with the error of the method. The widest range of room temperature and barometric pressure was 18—24° C and 740—780 mm Hg, respectively. BTPS values may be obtained by multiplying the volumes in this paper by a factor of 1.08—1.12.

[1] The terminology used here is in accordance with that recommended by English respiratory physiologists (4).



A B

Fig 1 Vital capacity (VC) in relation to age. A. males, B. females.



A B

Fig 2. Forced expiratory volume in one second ($FEV_{1.0}$) in relation to age. A. males, B. females.

bell damps the oscillations which tend to arise inside the bell.

The spirometer used was equipped with a plexiglass bell weighing 200 g with a diameter of 25.5 cm. The lateral pressure at the mouth, measured both with a strain gauge and a water manometer was less than 0 5 cm of water at maximal flow rates (6—7 l/sec.) No carbon dioxide absorber was used

The spirometer used in the study of children had somewhat smaller diameters of the connecting tube and the spirometer bell (3)

The curves were recorded on a motor driven kymograph drum with two standard speeds 5 and 50 mm/sec. respectively The volume changes were recorded by means of a pen attached to the counter-balance and by means of a ventilograph, which recorded only the inspiratory movements of the spirometer geared down ten times, producing a stepped curve at continued breathing

The volume factors were approximately 50 and 27 ml/mm for the two types of spirometers. The error of measurement was about 0.5 mm. This means for the vital capacity an error of 0 5—1 % and for the maximum voluntary ventilation an error of 0.5—2

Procedure

The subjects were studied in a sitting position and were thoroughly informed of the procedure. They were instructed to press the rubber mask gently but firmly against the face and to breathe through the wideopen mouth. Each recording was started with a few quiet breaths to let the patient settle

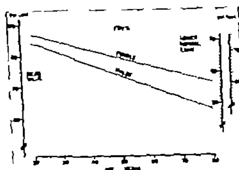


Fig. 5 Nomogram for predicted FEV %. Note that the scales for lower normal limit are different for males and females.

when VC and FEV were rectilinearly correlated to age and height. No improvement in R. S D was obtained when using more complicated functions, as *e. g.* the cube of the height. For the FEV $_{\%}$ the only significant correlation found was to age the best expressions are the straight lines shown in the nomograms of fig. 5

Table II gives the simplified equations for the adults. To permit an easy calculation of predicted normal values for VC, FEV and FEV $_{\%}$ nomograms have been constructed. In these nomograms both the mean value and the lower limit of normal (mean value — 2 S D) have been included (fig 3—5)

Discussion

When the two groups of adults were compared a slight but statistically significant difference between means was found for both VC and $FEV_{1.0}$. No such difference was found for the lower normal limits. The two materials are indicated by different symbols in fig 1 and 2. Both groups fit well into the general equation described above. Since the technical details are identical when performing the tests, the only possible explanation of this difference would be the selection of cases. A true random selection of a material of this size is very difficult to obtain and the inclusion of both groups was considered justifiable.

The maximum volumes seem to occur between the ages of 25 and 35 years (see also the statistical appendix) after the age of 35 there is a steady decline. This is in general agreement with the findings of Robinson (12) and Needham et al. (11) The present material is the largest one published covering such a wide range of age of both sexes. It should be pointed out that this material represents a cross-section of a population of different ages. This does not necessarily imply that it represents the true change with ageing in an individual It is, for instance well known that the body height for a given age is increasing

It is evident from fig 1 and 2 that the variations in both VC and $FEV_{1.0}$ within a population varying in age from 7—70 years can be described by single equations. However such equations would be difficult to handle in clinical routine. Statistical analysis of our results showed that separation of children and adults permitted simplified equations without loss of predictive accuracy

As the purpose of this study was to obtain data permitting a calculation of predicted normal values, nomograms were constructed, including both predicted mean value and predicted lower limit of normal. *It is of great importance*, when using such predicted values, to remember the fairly large biological variation around the mean. This variation amounts to 15—

These nomograms can be obtained from the Department of Clinical Physiology Sahlgrenska sjukhuset, Göteborg SV Sweden.

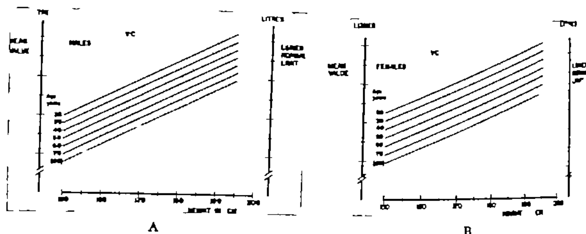


Fig 3 Nomogram for predicted vital capacity (VC) in A. males, B. females.
How to use Project vertically from height to interpolated age line. Read off horizontally to mean normal value at left and lower normal limit at right.

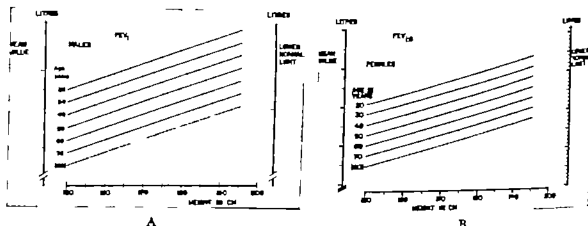


Fig 4 Nomogram for predicted forced expiratory volume in one second ($FEV_{1.0}$) in A. males, B. females.

Results

The individual values of VC and $FEV_{1.0}$ for both males and females are plotted against age in fig 1 and 2 Equations describing the accumulated data are given in table I These equations are illustrated in the figures the heavy lines indicate the mean function of the respective volumes with increasing age in the population. The dotted lines represent the 95 % confidence limits of the same functions. The early part would probably be better represented by a sigmoid line, since the relationship between body height and age is most pronounced in this age group A special account of the statistical treatment is given in the appendix (p 191)

Since the use of these equations would become very cumbersome in a daily routine, the material was divided into two parts, one comprising the ages 7—19 and the other 20—70 years. Simplified equations could then be used as equally good approximations of the spirometric functions with regard to sex age and height. The younger age group is dealt with in detail in a separate report (3)

The statistical analysis of the adult material revealed that the residual standard deviation (R. S D) was smallest

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Statistical Appendix

By

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1. *Relationship between functions*

An analysis was made to study the possible interrelationship between various pulmonary functions (y-variables) and one or several of the x-variables, height, weight and age for the two sexes. This was done in order to evaluate the x-variables as predictors for the y-variables. If such interrelations can be shown, it is possible to determine more accurately the normal value for the y-variable, and its limits.

After plotting the interrelated x- and y-values the following types of function were tested:

y as a linear function of age, height and weight,

y as a function of the square of the height or the cube of the height and

y as a function of log age, log height and log weight.

In the first and the last instances the x-variables have been used separately and in various combinations.

CHOICE OF FUNCTION TYPE

The principle for choice of function type is briefly as follows. For each y-variable the mean value, $\bar{y}$ and the standard deviation around the mean value, S_y, were calculated. For each function type the regression coefficients and the standard errors of these were calculated, together with the residual standard deviation, $S_y\{x\}$ ({x} is a vector containing one or mor

Table III Comparison between present and previous materials with the same type of regression equations

	Males	R.S.D.
Goldman & Becklake (5)	VC = −5.335 − 0.031 A + 6.40 H	0.49
Kory et al. (9)	VC = −3.60 − 0.022 A + 5.20 H	0.58
Needham et al. (11)	VC = −1.910 − 0.035 A + 4.331 H	0.44
Present material	VC = −2.212 − 0.020 A + 4.806 H	0.50
Kory et al. (9)	$FEV_{1.0}$ = −1.59 − 0.028 A + 3.70 H	0.5.
Present material	$FEV_{1.0}$ = −1.00 − 0.033 A + 3.44 H	0.50
	Females	
Goldman & Becklake (5)	VC = −4.360 − 0.018 A + 5.20 H	0.43
Needham et al. (11)	VC = −2.710 − 0.020 A + 3.937 H	0.36
Present material	VC = −2.351 − 0.022 A + 4.036 H	0.40

A = age in years H = height in metres.

20 % of the mean value. The inclusion of a "lower limit of normal" will permit the delineation of abnormality with a high degree of accuracy (97.5 %). It should be emphasized however that 68 % of a normal population should show values within ± 1 S.D. and only 16 % should fall below −1 S.D. A subject showing values below −1 S.D. has only one chance in six of being normal.

When calculating the lower limit of normal the assumption was made that the numerical distance to the mean value was constant. This implies that the relative deviation from the normal value must be larger in subjects with a low VC or $FEV_{1.0}$ (small and old subjects) than in subjects with a high value (tall and young). This assumption was shown to be mathematically correct but might lead to misinterpretation when judging a low value in small and/or old subjects.

A comparison of the present series has been made with others using the same type of equation (table III) (5, 9, 11). It is apparent that the effect of age and height on the VC and FEV_1 is of similar magnitude in all studies. The differences in mean values, whether significant or not, are accordingly of less importance and presumably due to variations in case selection. The large material reported by Jouasset (8) is difficult to interpret statistically.

In all reported materials there is a slight decline of FEV $\%$ with increasing age. In the present material this decline appears large enough to be considered in clinical work.

Summary

296 males and 201 females, 7—70 years old, have been studied with identical techniques in three different laboratories in Göteborg.

Vital capacity and forced expiratory volume in 1.0 sec (VC and $FEV_{1.0}$) were plotted against age. Both could be described mathematically as a function of age and height for the whole range. Simplified equations and nomograms for VC and FEV_1 were deduced as rectilinear correlations with age and height for the adults. For the FEV % the only significant correlation was found with age.

Acta Medica Scandinavica. Vol. 173, fasc. 2, 1963

From the Chest Clinic, the University Göteborg, Sweden

Spirometric Studies in Normal Subjects

II. Ventilatory Capacity Tests in Adults

By

G. Birath, I. Kjellmer and L. Sandqvist

The introduction of improved methods for measuring airflow has changed the focus of interest from static lung *volume* measurements to dynamic lung *ventilation* measurements. These estimations seem to be of great value in clinical practice. For their proper use, however, the predicted figures have to be as correct as possible i.e. calculated from examination of the population in question and performed in a well defined manner on a suitable spirometer.

The aim of this study was twofold:

1. To secure standard values for three different ventilation tests, obtained on healthy and active men and women of different ages, using a spirometer specially designed for dynamic studies, and

2. To compare the results from these ventilation tests with one another.

The ventilation tests chosen were the *forced expiratory volume* in 1.0 sec. ($FEV_{1.0}$), *the maximal midexpiratory flow* (MMF) and *the maximum voluntary ventilation* with a fixed *frequency of 40 RPM* (MVV_{40}).

In part presented before the Swedish Association for Clinical Physiology February 6, 1959.

Submitted for publication July 17, 1962.

Material

Three age groups of subjects were examined: 20—25, 40—45 and 60—65 years of age. In each age group both men and women were studied. The physical characteristics of the material are shown in table 1. The youngest age group is mainly composed of students and nurses, while the older groups contain factory workers, office employees and housewives.

Only those subjects who had no history of pulmonary or cardiac diseases were accepted. Special care was taken to exclude subjects with long or repeated cough periods, with morning expectoration or with complaints of a degree of dyspnoea abnormal for their age. In the males about one third were non-smokers, one half smoked less than 10 cigarettes a day and one sixth smoked more than 10 cigarettes a day. In the female group more than two thirds were non-smokers, one fifth smoked less than 10 cigarettes a day while only three subjects smoked more than 10 cigarettes a day.

All had a normal chest roentgenogram and showed normal movements of the diaphragm on fluoroscopic examination. In about one third of the material there was no opportunity of fluoroscopy for the examination of diaphragm movements — instead two films were

Supported by a grant from the Swedish National Association for Heart and Lung Diseases.

of the variables X_1 = age, X_2 = height, X = weight, etc.

The regression coefficients were tested for significance. Necessary conditions for use of the regression function was that the coefficients were significantly different from 0 (level of significance = 5 %)

However the significance test is insufficient for the use of the interrelation function. The magnitude also of the regression coefficient is of importance. The x-factors in the regression function may be viewed as correction factors, and as such, must be of such a magnitude that the correction is meaningful. To illustrate this, assume that the coefficient for the x variable age is 0 0002 in a linear interrelation function with VC A change of age with 50 years would then imply a correction of the VC value by 0 01 this correction is meaningless in comparison with the accuracy of the method. Such a variable may therefore be excluded, though the interrelationship is statistically significant.

That function type has been chosen which gives the smallest residual standard deviation. Of two or more function types similar in these aspects, the simplest ones have been used. This was done to simplify the calculation As indicated above, the difference between the total standard deviation and the residual standard deviation must exceed a certain magnitude for a significant relationship to be chosen.

2 *Calculation of normal limits*

After having chosen the interrelated functions to be used, we examined whether the residual standard deviation was equally large for different values of the x-variables, i e whether the variation coefficient can be considered to be homogeneous. This was found not to be the case for the functions studied. However the standard deviations were found to change only slightly for different values of the x variables, and therefore the numerical value of the standard deviation varied less than did the variation coefficient. Lower and upper limits of normals were found by subtracting or adding two standard deviations. Outside each of these normal limits only 2.5 of normal individuals could be expected

3. *The combination of the three materials*

When constructing a mathematical model for the interrelationship between lung volumes and the independent variables height and age, the following assumptions were made.

1 The lung volume values increase with age, until a maximum is reached, and then decrease without reaching negative values.

2. The lung volume values increase, within a fixed age, with increasing height.

A theoretical model, that fulfills these assumptions, is

$$y = a\,x^{\beta}\,e^{\gamma} \quad e^{\lambda x}$$

where y = lung volume value x = age z = height. Some objections can be made to this model, since it does not include the co-variation between age and height. Such a co-variation should however be treated as a function of age which would unnecessarily complicate the model. Since height changes more rapidly in the younger age groups, a wider scatter is to be expected in that area with the model chosen.

The method of least squares was used to determine the function coefficients describing the lung volume as a bivariate function of age and height. These functions may be illustrated as two parametric functions (table I) In order to illustrate the adaptation of lung volumes to age, a deviation of the mean value function was made as follows For a given age, x, the mean value was calculated,

$$y = \int_{H_{min}(\ )}^{H_{max}(\ )} f(xz)\,dz$$

Here $H_{min}(x)$ and $H_{max}(x)$ respectively limit the usable range of heights for the respective age The points thus obtained have been adapted to the function $y = a \quad V^{\beta} e \quad V e^{\delta}$ After determination of the coefficient, this function has been illustrated as the curve drawn in heavy line in fig 1 and 2.

This method also makes it possible to determine the 95 confidence limits common to all ages. This approach gives a seemingly rather large interval for normal subjects, which probably is partly due to the selection of cases.

Derivation of the function with regard to x gives maxima at certain ages. For each lung volume there is a difference between the age of maximal level between the two sexes. This difference amounts to approximately 6 years, the women reaching their maximum earlier The implications of this are uncertain.

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That function type has been chosen which gives the smallest residual standard deviation. Of two or more function types similar in these aspects, the simplest ones have been used. This was done to simplify the calculation. As indicated above, the difference between the total standard deviation and the residual standard deviation must exceed a certain magnitude for a significant relationship to be chosen.

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This method also makes it possible to determine the 95 % confidence limits common to all ages. This approach gives a seemingly rather large interval for normal subjects, which probably is partly due to the selection of cases.

Derivation of the function with regard to x gives maxima at certain ages. For each lung volume there is a difference between the age of maximal level between the two sexes. This difference amounts to approximately 6 years, the women reaching their maximum earlier The implications of this are uncertain.

Table II. Mean values, standard deviations and ranges of obtained data

		Males			Females		
		$FEV_{1.0}$ (l)	MMF (l/sec)	MVV_{40} (l/min)	$FEV_{1.0}$ (l)	MMF (l/sec)	MVV_{40} (l/min)
20—25	Mean	4.45	4.89	152.8	3.19	4.26	102.9
	S. D.	0.42 (9.4)	0.93 (19.4)	15.0 (9.8)	0.38 (11.9)	0.73 (17.6)	12.0 (11.6)
	Range	3.72—5.51	3.42—6.58	127.1—177.3	2.66—4.02	3.08—5.71	79.2—118.7
40—45	Mean	3.45	3.25	123.6	2.82	3.58	91.6
	S. D.	0.67 (19.5)	1.08 (33.3)	21.8 (17.7)	0.34 (12.1)	0.62 (18.4)	13.7 (15.0)
	Range	2.75—5.64	1.91—6.55	97.2—183.9	2.23—3.39	2.46—4.49	72.8—121.0
60—65	Mean	2.98	2.79	101.8	2.07	2.09	67.5
	S. D.	0.41 (13.8)	0.97 (34.8)	15.8 (15.5)	0.39 (18.9)	0.81 (38.8)	12.1 (17.9)
	Range	2.33—4.02	0.99—4.52	74.1—146.5	1.31—3.02	0.70—3.35	44.8—95.3

The numbers in parentheses are S.D. calculated as % of mean value (coefficient of variation)

All values are ATPS.

Table III. Regression equations

Males	MMF = 5.85 — 0.0323	age in years.	R. S. D = 1.00
	MVV_{40} = 180.5 — 1.288	age in years.	R. S. D = 16.6
Females	MMF = 5.63 — 0.0579	age in years.	R. S. D = 0.71
	MVV_{40} = 113.1 — 0.618	× age in years.	R. S. D = 15.1

R. S. D = residual standard deviation.

physiological respiratory rate (1, 2). Further 40 is sufficiently low frequency to permit even patients with severe degrees of obstructive lung disease to perform the test.

When evaluating normal material, it is important to know the error of the determination of the various factors in single individual. Some investigators have stated what maximum differencies could be allowed in duplicate determinations of the vital capacity (Miller et al. (10) — 100 ml. Kory et al. (7) — 5 %) and of the maximum voluntary ventilation (Miller et al. (11) — 5 l/min.). In the present investigation the volumes were not read directly from the spirometer but were calculated from the recorded curves. The difference between duplicate checks could thus not be determined during the recording procedure and instead, the difference between the highest and the second highest value for each test has been calculated. This difference amounted to 1—3 % of the current factor except for the MMF where it was 3—7 %. It never exceeded 4 % for the $FEV_{1.0}$, 6 % for the MVV_{40} and 10 % for the MMF. Thus the order of magnitude of this difference usually was about the error of measurement taken twice and never exceeded this error taken four times, except for the MMF where it could be as great as 5 to 10 times the error of measurement.

Results

Mean values, standard deviations, ranges and coefficients of variation for $FEV_{1.0}$, MMF and MVV_{40} are given in table II.

A significant difference between sexes was established and a significant relation-

Table I Physical characteristics of the material

Group	Sex	No.	Age		Height			Weight		
			Mean	Range	Mean	S.D	Range	Mean	S.D	Range
20—25	♂	20	22	20—24	1.80	0 05	1 70—1.89	68.4	6.5	57—80
	♀	20	21	20—23	1.66	0 04	1.58—1 74	57.6	4.8	49—64
40—45	♂	21	43	39—45	1 76	0.07	1.62—1.91	76.8	8.4	58—85
	♀	20	41	39—45	1 65	0.06	1.55—1 74	62.9	7 4	54—81
60—65	♂	21	62	61—66	1 71	0.07	1 61—1.84	77 1	13.0	55-111
	♀	18	62	58—66	1.63	0 04	1.54—1 71	70.0	8.2	54—84

taken, one postero-anterior and one lateral to exclude subjects with shallow or obliterated sinuses.

Methods

The spirometer used was of the low-resistance type constructed by Bernstein et al (4) slightly modified as described in a preceding paper in this issue (3)

FEV$_{.4}$. The procedure for recording FEV$_{.4}$ is described in the same paper (3)

MMF This test may be defined as the mean flow rate during the two middle fourths of the forced expiratory vital capacity expressed in l/sec., see Leuallen and Fowler (8) The MMF values were obtained from the ordinary forced vital capacity curves, thus from *the same curves as were the FEV*$_{.4}$ values.

To deduce the MMF the FVC was divided into four equal volumes, and the time taken to expire the two middle volumes was measured. The MMF was then calculated as the mean flow over this time period

In each subject the MMF was measured on the four best FVC curves. Each subject is represented by its maximum value As a rule, the maximum values for MMF and FEV were both found on the curve with the greatest FVC value.

MVV$_{40}$. The subject was instructed to breathe as deeply as possible for a period of at least 15 sec., following the beats of a metronome. The test was repeated until the subject seemed to have reached his maximum. As a rule 3 to 5 curves were recorded. Tests where the frequency differed more than 2 RPM from 40 were discarded. In the majority o cases the subjects had no difficulty in keeping the frequency within the limits given.

Discussion of methods

The maximum voluntary ventilation may be performed at either a free or a fixed frequency The majority of previous investigators have used free frequencies. In the present study a fixed frequency has been used, giving the following advantages

(1) as the MVV value depends upon the frequency chosen (4) the dispersion at a fixed frequency must be smaller than the dispersion at frequencies varying from *e.g.* 30 to 180 RPM.

(2) the fixed frequency allows of more accurate comparison of values obtained from one individual at different occasions. The latter is perhaps the more important advantage.

(3) the so-called free respiratory rates are certainly often not chosen by the patient but by the laboratory assistant guiding the examination. Under such circumstances it is better to know what frequency is used.

A disadvantage, however is that a fixed frequency may interfere with a patient's normal pattern of breathing, since a patient with restrictive ventilation insufficiency is known to choose a high respiratory rate while a patient with obstructive ventilation insufficiency breathes at a *low rate* during forced hyperventilation. Some patients also (especially old and poorly ones) may show more difficulties in performing the test at a fixed than at a free frequency

40 RPM was chosen as the fixed frequency for the following reasons. 40 RPM may be taken as a reasonable value of the maximum

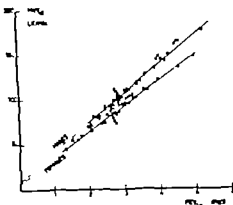


Fig. 3. Correlation between MVV_{40} and $FEV_{1.0}$. Regression equations and correlation coefficients (r)
Males $MVV_{40} = 31.65$ $FEV_{1.0} + 11.41$; $r = 0.91$.
Females $MVV_{40} = 29.54$ $FEV_{1.0} + 7.96$ $r = 0.89$.

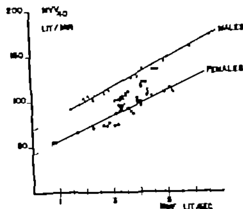


Fig. 4. Correlation between MVV_{40} and MMF. Regression equations and correlation coefficient (r)
Males $MVV_{40} = 15.60 \times MMF + 69.19$ $r = 0.71$
Females $MVV_{40} = 15.77 \times MMF + 42.87$ $r = 0.78$.

variation coefficients for $FEV_{1.0}$ and MVV_{40} are 14.3 and 14.6 respectively while the same value for MMF amounts to 27.1. Leuallen and Fowler (8) and Kory et al. (7) report similar values. This great variation coefficient must be regarded as a considerable drawback when evaluating a single test result, as it implies a prediction error that is about twice as high as that for $FEV_{1.0}$ and MVV_{40}.

It should be pointed out, however that the MMF test cannot be directly compared to the FEV or the MVV_{40} tests, as the flow rate of different parts of the respiratory cycle is determined by different factors. Hyatt et al. (6) have inferred that the shape of the last part of the expiratory flow curve is mainly determined by resistance in the lower airways, while the shape of the first part (i.e. at lung volumes near maximum inflation) is determined by several factors such as muscular effort, motivation and airway resistance. This might indicate that MMF is a more specific test of lower airway resistance than are the other tests. Three observations in this study may support the view that these tests depend on different variables. (1) There is less difference between male and female values for MMF than for the other tests, indicating that muscular power may be of little consequence for the MMF values. (2) The correlation between MVV_{40} and FEV is quite strong (fig. 3) while that between MVV_{40} and MMF is considerably weaker (fig. 4) and (3) the male and female groups behave similarly when correlating MVV_{40} and $FEV_{1.0}$, while there is a striking difference between the regression lines of the sexes when correlating MVV_{40} and MMF.

To test whether the MMF might be a more specific test of lower airway resistance than are the other two, 34 patients were investigated using the three tests. These patients were selected in the following way. In a patient material the FEV % values were compared with the results from the single breath nitrogen elimination test (9). Of the 264 patients studied

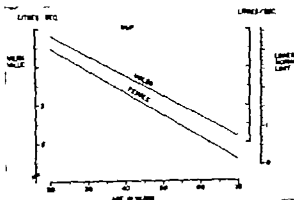


Fig 1 Nomogram for MMF in relation to age. Note that the scales for lower normal limits are different for males and females.

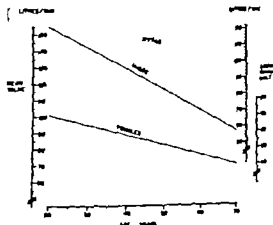


Fig 2 Nomogram for MVV_{40} in relation to age. Note that the scales for lower normal limits are different for males and females.

ship to age was also found for all three tests ($P < 0.01$) as well as to body height for $FEV_{1.0}$ and MVV_{40} ($P < 0.05$) Estimating equations and nomograms for $FEV_{1.0}$ are given in another paper in this issue (3)

It is true that there is a statistically significant relationship between MVV_{40} and body height but this was shown to be of minor practical importance since the multiple regression coefficient rose insignificantly when body height was excluded from the estimating equations. This is a parallel to the findings of Goldman and Becklake (5) see also Statistical methods in reference (3) Therefore only the regression on age is given (table III) In fig 1 and 2 are given nomograms for MMF and MVV_{40} where both the predicted mean value and lower normal limit (97.5 % confidence) can be read[1]

In fig 3 and 4 is shown the relationship between MVV_{40} and $FEV_{1.}$ as well as between MVV_{40} and MMF

All equations were calculated by the method of least squares.

[1] These nomograms can be obtained from the Department of Clinical Physiology Sahlgrenska sjukhuset, Göteborg SV Sweden.

Discussion

Comparison with other materials

MMF Normal values for MMF were published by Leuallen and Fowler when they introduced this test of ventilation in 1955 (8) Our values correspond closely to theirs except for the youngest age groups, where the present material reaches somewhat higher values. The only other study on the MMF to our knowledge has been reported by Kory et al. (7) They only give the mean value for the whole material which value agrees well with the values found here.

MVV_{40} Any comparison of MVV must take the frequency into consideration. Most previous studies have used free frequencies. As the subjects having their choice as a rule choose higher frequencies than 40 RPM and moreover are usually encouraged by the investigator to increase their respiratory rate still more no comparison between such investigations and the present one seems justified

Comparison of tests studied

As can be seen from table II the variation coefficients for MMF in the various groups are about twice as high as those for the other tests in this study The mean

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From the Department of Clinical Physiology University of Göteborg, Sweden

Spirometric Studies in Normal Subjects

III. Static Lung Volumes and Maximum Voluntary Ventilation in Adults with a Note on Physical Fitness

By

G. Grimby and B. Söderholm

Normal values for dynamic spirometry have been reported in a previous article (3) The aim of this study is 1) to supplement data on static lung volumes on the same subjects reported above and 2) to secure standard values for maximum voluntary ventilation when a free frequency was used. The physical working capacity was studied in the male subjects and compared with the performance of different spirometric tests.

Material

One hundred and fifty tw men between 20—65 and 58 women between 18—72 years of age were studied. The age reached t the birthday of the year of investigation was used. Mean and standard deviation (S. D.) of age, height and weight are given in table I.

Most of the male subjects belonged to employment groups undergoing yearly physical check up (naval officers, clerks, foremen, bakers and medical students) Physical examination, chest X-ray and an 11-leads ECG t rest were done in l male subjects. A work test with recording of ECG was performed in 115 subjects.

In the female group only a small number were derived from the yearly health check up (clerks and telephone operators) Most subjects were house wives who belonged to different organizations and volunteered the study They had no history of heart or lung disease and fluoroscopy of the chest was nor mal. In no subject there was found any evidence of heart or lung diseases.

Methods

Functional residual capacity (*FRC*) was meas ured with the subject comfortably seated in an armchair tilted slightly backward. The helium dilution technique was used as describ ed earlier (12) The error of a single deter mination was then found to be 180 ml. This corresponded to an error of 7.5 % at func tional residual capacity averaging 2.4 l. Only single determinations were performed. At the end of this determination maximal expira tion was performed for the calculation of the *residual volume* (*RV*).

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130 had pathologically increased values for nitrogen elimination i.e. had signs of a disturbed distribution of inspired gas. Of these 130 patients 96 simultaneously had a pathological FEV % value. The remaining 34 patients, thus, had normal FEV % values but pathological nitrogen elimination tests. In this group of patients it might be expected that several had a mild airway obstruction this was revealed by the distribution test but was not severe enough to give a pathological FEV % value. It was thought that a more sensitive indicator of obstructive lung disease would give a considerably higher number of pathological values in this patient group. The result, however was that pathologically reduced values were reached for the FEV . in 12 cases, for the MVV_{40} in 18 cases and for the MMF in 4 cases only In these 4 cases, moreover both the $FEV_{1.0}$ and the MVV_{40} were severely reduced.

As judged from this, MMF cannot be considered to give any further information than can FEV and MVV_{40}. In this laboratory therefore, only the two last mentioned tests are used together with FVC and FEV % as routine tests for patient-studies. The advantage when using both $FEV_{1.0}$ and MVV . is that the cooperation of the patient can be controlled because of the good correlation between the two tests.

Summary

Dynamic lung function studies were performed on 120 healthy men and women aged 20 to 65 using a special low-resistance spirometer Normal values are given for the maximal midexpiratory flow (MMF) and the maximum voluntary ventilation with a fixed frequency of 40 RPM (MVV_{40}) The advantage of a fixed frequency is discussed MVV . and the forced expiratory volume in one second ($FEV_{1.0}$) show a good correlation to each other but a poorer correlation to MMF

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Addendum

Since th preparation of this manuscript FAIRBAIRN et al. (Thorax 17 168, 1962) have found "that the discriminatory power of the absolute value of the F E.V is at least as great as that of the M. M. F and exceeds that of the F E.V % and of every other test which we studied" They did not, however compare with the MVV test.

In the previous article (3) both the spirometer used and the performance and calculation of *FC*, $FEV_{1.0}$ and *FEV* ... have been described in detail.

Total lung capacity (*TLC*) was calculated as the sum of the vital capacity and the residual volume measured.

The *maximum voluntary ventilation* (*MVVF*) was determined at a frequency chosen by the subject. T ensure satisfactory cooperation, the procedure was explained to the subject who was urged to breathe as rapidly and deeply as possible for about 15 sec. In each case at least three tests were made. The highest value was taken and the ventilation expressed in l/min. All lung volumes and the maximum ventilation were given at A. T P S. No correction for a variation in temperature and saturation was used since actual measurements in the trachea have shown that temperature and saturation equilibrium does probably not occur (cf. 7) All spirometries were led by a trained nurse.

The physical working capacity was studied on an electrically braked bicycle ergometer (6) The subjects performed successively increasing work loads as described by a. o. Wahlund (13) The test was started at 300 kpm/min. Each work period lasted 4—6 min. after which the work load was increased in steps of 300 kpm/min., until beart rate of approximately 170 beats/min. was reached. Heart rate was calculated from the ECG A "steady state" was defined to exist when 2 countings with an interval of 2 min did not deviate more than ± 5 beats/min. If such steady state was not reached within 6 min. the previous work load was used as the highest steady state work load for the subject.

Results

T ble I shows mean values and standard deviations of the variables studied.

The results have been statistically analysed as described in the appendix of a previous article (3). Table II shows the regression coefficients and constants, which can be used to establish equations for calculation of normal values. These were derived for both sexes from multiple regression analysis with regard to age, height and weight. In the final calculation of the equations only those correlations which were statistically significant ($P < 0.05$) were included. The residual standard deviations (RSD) when employing these equations, are given in the last column of the table. The upper or lower limit of normal (outside which only 2.5 % of the normal population will be found) is calculated by adding or subtracting 2 × RSD from the value given by the equation. To simplify these calculations nomograms have been constructed) In these nomograms both the mean value and the limit of normal have been included (figs. 1—6)

Ex The normal value and upper limit for FRC are sought in a man, age 40 years, height 177 cm and weight 76 kg. The following equation gives the normal value

$$FRC = 40 \times 0.015 + 1\ 77 \times 5.40 - - 76 \times 0.037 - 3.89 = 3.28\ l$$

and the upper limit of normal equals

$$3.28 + 2 \times 0.56 = 4\ 40\ l$$

These values can also be obtained from the nomogram in fig 4 The intersection between height = 177 cm and weight = 76 kg is found in the lower left quadrant. This point is transposed to the line corresponding to age 40 years by use of the sloping grid. *Mean value* and *upper* normal limit are then read directly on the scales above.

Average heart rates and respiratory frequencies at different work loads are given in table III The subjects ha e been divided into groups according to the highest work load performed in a steady

These nomograms can be obtained from the Department of Clinical Physiology Sahlgrenska sjukhuset, Göteborg SV Sweden.

Table I Mean values and standard deviations (S D) of age, height, weight and spirometric functions studied

	Males		Females	
	Mean value	S. D	Mean value	S. D.
Age (yrs)	40.4	14.1	40.1	13.8
Height (m)	1.77	0.07	1.64	0.06
Weight (kg)	75.7	8.8	61.7	7.2
TLC (l)	6.65 ± 0.06	0.77	4.63 ± 0.09	0.69
VC (l)	4.89 ± 0.05	0.65	3.38 ± 0.08	0.59
FRC (l)	3.30 ± 0.05	0.66	2.16 ± 0.07	0.51
FRC/TLC (%)	50.0 ± 0.6	7.4	46.7 ± 0.7	5.1
RV (l)	1.75 ± 0.04	0.44	1.25 ± 0.05	0.35
RV/TLC (%)	24.4 ± 0.4	5.3	27.0 ± 0.8	5.8
MVV_F (l/min)	159 ± 3	34	107 ± 3	22
$FEV_{1.0}$ (l)	3.76 ± 0.06	0.68	2.76 ± 0.08	0.57
FEV (%)	76.7 ± 0.7	8.2	81.6 ± 0.9	6.5

TLC = total lung capacity VC = vital capacity FRC = functional residual capacity
RV = residual volume. MVV_F = maximum voluntary ventilation with free frequency
$FEV_{1.0}$ = forced expiratory volume in one second. FEV % = $FEV_{1.0}$ in percentage of VC.

Table II Regression equations and residual standard deviations (R.S D) for each lung volume measurement

	Sex	Regression coefficients			Constant	R.S.D.
		Age (yrs)	Height m	Weight (kg)		
TLC (l)	♂	—	+6.92 ± 0.98	−0.017 ± 0.007	− 4.30	0.67
	♀	−0.015 ± 0.005	+6.71 ± 1.14	—	− 5.77	0.48
VC (l)	♂	−0.020 ± 0.004	+4.81 ± 0.66	—	− 2.81	0.50
	♀	−0.022 ± 0.004	+4.04 ± 0.95	—	− 2.35	0.40
FRC (l)	♂	+0.015 ± 0.005	+5.30 ± 0.83	−0.037 ± 0.006	− 3.89	0.56
	♀	—	+5.13 ± 1.05	−0.028 ± 0.008	− 4.50	0.41
FRC/TLC (%)	♂	+0.18 ± 0.03	—	−0.12 ± 0.03	+ 52.3	6.8
	♀	+0.16 ± 0.03	—	−0.08 ± 0.02	+ 45.2	4.7
RV (l)	♂	+0.022 ± 0.003	+1.98 ± 0.56	−0.015 ± 0.004	− 1.54	0.38
	♀	+0.007 ± 0.003	+2.68 ± 0.75	—	− 3.42	0.33
RV/TLC (%)	♂	+0.33 ± 0.05	—	−0.14 ± 0.03	+ 23.4	4.3
	♀	+0.28 ± 0.04	+27 ± 7	—	− 28.0	5.5
MVV_F (l/min)	♂	−1.42 ± 0.24	+79.0 ± 39.1	—	+ 76	30
	♀	−0.77 ± 0.19	—	—	+138	20
$FEV_{1.0}$ (l)	♂	−0.033 ± 0.04	+3.44 ± 0.66	—	− 1.00	0.50
	♀	−0.028 ± 0.04	+2.67 ± 0.85	—	− 0.54	0.36
FEV (%)	♂	−0.37 ± 0.05	—	—	+ 91.8	7.2
	♀	−0.26 ± 0.05	—	—	+ 92.1	5.4

600 kpm/min	900 kpm/min		1,200 kpm/min	
Respiratory rate	Heart rate	Respiratory rate	Heart rate	Respiratory rate
24.0 ± 1.0	—	—	—	—
4.9	—	—	—	—
22.5 ± 0.5	154.9 ± 2.1	27.5 ± 0.6	—	—
4.1	15.9	3.2	—	—
21.0 ± 0.5	144.6 ± 1.7	23.6 ± 0.6	168.6 ± 1.4	30.1 ± 0.9
3.1	11.1	3.7	9.2	5.6
22.1 ± 0.4	150.5 ± 1.5	25.1 ± 0.5	168.6 ± 1.4	20.1 ± 0.9
3.8	14.9	4.8	9.2	5.6
115	100	100	43	43

heart rate at submaximal work loads and spirometric data. This is taken as an indication that differences in physical fitness will not limit the use of the normal values predicted from this material as long as extreme variables are not included.

When comparing the results of previous spirometric studies in normal subjects large variations are observed. These are probably to a very large extent dependent upon the selection of subjects but also upon the spirometer used. The latter factor is specially important for the dynamic function tests.

A valid comparison of predicted lung volumes can only be made when identical mathematical models have been used. In such cases the regression coefficients for age, height and weight etc. could be directly compared. This has not been feasible since most authors have used different models without stating why the specific model was preferred. In the present investigation many different models were tested and the one giving the least standard residual deviation was chosen (cf. Statistical appendix (3)). No improvement in prediction was found when the square or cube of height was used alone or in combination with other parameters.

In dynamic function tests a low resistance to respiration is of utmost importance (4). It is probable that the low maximum breathing capacities reported by Baldwin et al. (2) and Needham et al. (11) depend upon a high resistance to breathing. Later studies, performed with an improved spirometer system as those of Miller et al. (10) and Kory et al. (9) show higher maximum voluntary ventilations which are similar in magnitude to those of the present investigation.

Maximum voluntary ventilation can be performed either at a fixed frequency or at a frequency chosen by the subject. The choice between the two techniques is to some extent dependent upon the aim of the study. As a screening test MVV will give as good information about disturbances of the mechanics of respiration etc. as MVV at a fixed frequency even though the repeatability at a fixed frequency seems to be higher (5).

There was no correlation between MVV_F and the physical working capacity as judged from the heart rate at sub-

Table III Heart and respiratory rates during exercise in males

		300 kpm/min		600 kpm/min
		Heart rate	Respiratory rate	Heart rate
"600-group" (n = 15)	Mean	112.2 ± 3.1	20.0 ± 0.9	143.5 ± 3.9
	S. D.	12.2	3.4	15.0
"900-group" (n = 57)	Mean	104.1 ± 2.0	20.2 ± 0.5	129.7 ± 2.1
	S. D	15.3	3.5	16.1
"1,200-group" (n = 43)	Mean	96.8 ± 1.8	19.4 ± 0.4	118.8 ± 1.5
	S. D	12.0	2.7	10.0
All subjects	Mean	102.5 ± 1.4	19.9 ± 0.3	127.4 ± 1.5
	S. D.	14.6	3.2	15.9
	n	115	115	115

n = number of subjects.

state. A linear correlation between heart rate and work load was found in all subjects At the same submaximal work load no difference in heart rate with age was found Respiratory frequency however did not show any such correlation to work load The increase in respiratory frequency was higher when the subject started working on the final work load than on the previous work load This difference in increments of respiratory rate is significant both for those performing 1,200 kpm/min and for those performing 900 kpm/min ($P < 0.001$ and $P < 0.05$ respectively)

When heart rate at a certain work load was correlated to either VC, MVV_F or FEV_1 no significant relationship was found Nor was there any significant difference in these spirometric data between the two groups performing 900 and 1 200 kpm/min This is in accordance with Åstrand (1) who found no correlation between $FEV_{1.0}$ and maximal oxygen uptake in a group of normal females.

In the present material, there was a significant correlation ($r = -0.4$ $P < 0.01$) between the respiratory frequency during work and $FEV_{1.0}$ indicating a higher respiratory rate in subjects with a low $FEV_{1.0}$.

Discussion

In the present study efforts were made to include groups differing both in economical standards and in physical activity in daily life.

The importance of a broad basis in the selection of normal individuals is apparent from the slight but significant difference in mean values for VC and $FEV_{1.0}$ in the two groups described in the earlier article (3) Varying degrees of physical fitness might be an important factor for such differences.

Work tests were performed in order to characterize the male population and to analyse the possible effects of physical fitness on spirometric functions. The physical working capacity of this population seems, both in range and distribution, to be quite comparable to that reported by Frisk et al. (6) No relationship was obtained however between physical working capacity as judged from the

maximal work loads. Ordinarily only some 60—70 % of the predicted maximum voluntary ventilation is used even at maximum work loads (1). Thus if the ventilatory capacity is a *limiting* factor for the physical working capacity this will be obvious irrespective of the technique used.

When the subject is urged to a maximum ventilatory performance he usually choses a rather high frequency and in the present series the respiratory rates were 82 (S. D = 21) and 84 (S. D = 17) for the males and females respectively. This is in agreement with Bernstein et al. (4) who showed that the optimum frequency was above 70 r. p. m. The frequency chosen was independent of sex or age.

A good cooperation between the investigator and the test subject is important when evaluating dynamic function tests. In practice comparison of two independently performed tests is of value to control such cooperation. This is made possible by the good correlation between MVV_F and $FEV_{1.0}$, which has been shown repeatedly. In the present series the following equations have been established.

for males $MVV_F = 28.6 + 34.3 \times FEV_{1.0}$

S. D = 24.9 r = 0.70

and for females $MVV_F = 23.7 + 30.3 \times FEV_{1.0}$

S. D = 14.4 r = 0.77

This relationship however does not hold when ventilation is impaired by e. g. stenoses of the upper airways as in cases of tracheal stenosis or paresis of the vocal cords. In such cases $FEV_{1.0}$ can be fairly normal while MVV_F is markedly reduced as a result of the increased resistance to inspiration.

Summary

Static lung volumes and dynamic function tests have been studied in normal men and women. Equations, and nomograms for *TLC*, *FRC* and MVV_F, for the prediction of normal values and their limits are presented.

In the male group physical fitness was studied on a bicycle ergometer. No correlations were found between spirometric data and the working capacity as judged from heart rate at submaximal work loads.

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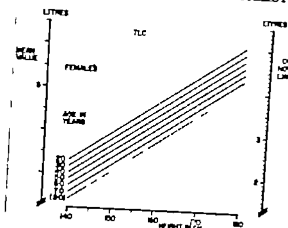


Fig 1 Nomogram for the prediction of total lung capacity (TLC) in women.

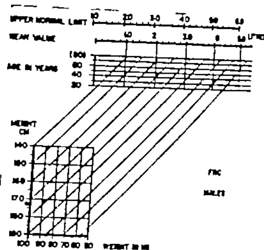


Fig 4 Nomogram for the prediction of functional residual capacity (FRC) in men. See explanation for use in text.

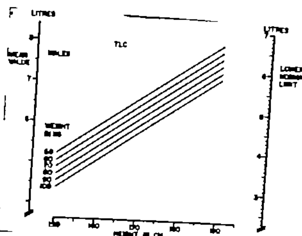


Fig 2. Nomogram for the prediction of total lung capacity (TLC) in men.

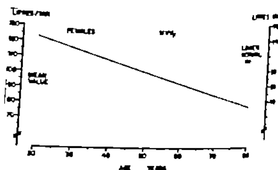


Fig 5 Nomogram for the prediction of maximum voluntary ventilation (MVV F) in women.

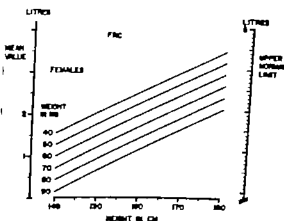


Fig 3. Nomogram for the prediction of functional residual capacity (FRC) in women.

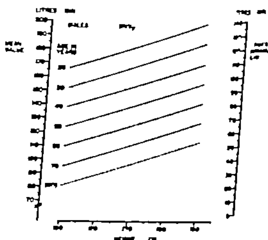


Fig 6. Nomogram for the prediction of maximum voluntary ventilation (MVV F) in men.

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From Sahlgrenska Sjukhuset and the Department I of Pathology
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Primary Endocardial Fibroelastosis in an Adult

By

Osborne Bartley, Per Björntorp, Folke Knutson, Olav Thulesius
and Edvardas Varnauskas

Endocardial fibroelastosis is an uncommon disease. At the present state of our clinical knowledge it has not, as a rule, been possible to diagnose the condition during life. At autopsy one finds enlargement of the heart with pronounced, whitish thickening of the mural endocardium, often in combination with myocardial hypertrophy. Microscopically there is beneath the intact endothelium a hyalinofibrotic thickening of the endocardial lining with numerous elastic fibrils disposed parallel to the surface.

Two forms of the disease are recognized (8). A secondary variety — where the lesions are confined to discrete patches — occurs concomitantly with various cardiovascular disorders, such as congenital malformations of the heart, valvular abnormalities, especially those in the aortic valves, hypertension, diseases of the coronary vessels and myocarditis of different types. But there exists also a primary or idiopathic variety of endocardial fibroelastosis in which the sole manifest cardiac alteration is a diffuse lesion of the endocardium in several cavities. The idiopathic variety of endocardial fibroelastosis is uncommon in infants and a rarity in the adult. Exception should perhaps be made for that form of primary endocardial fibroelastosis which is endemic in the tropics, particularly in tropical Africa, where it is regarded as one of the commoner causes of heart failure (3).

The etiology is unknown; numerous pathogenetic hypotheses have been advanced but none has been widely accepted. The problems of endocardial fibroelastosis have recently been surveyed by Thomas et al. (12), Black-Schaffer (1), Lynch and Watt (7), Lambert and Vlad (6) and Lehndorff (8).

In the following a case of primary endocardial fibroelastosis in an adult with lesions in all cardiac cavities will be reported, whereupon the differential diagnosis and the outlook for treatment will be discussed.

Case report

The patient, a female bookshop attendant aged 58, had previously been in good health, apart from rubeola at age 7 and sore throat and temperature of 40° C for 4 days at age 53. Her hereditary and social hygienic backgrounds were unilluminating.

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The patient, a female bookshop attendant aged 38, had previously been in good health, apart from rubeola at age 7 and sore throat and temperature of 40° C for 4 days at age 33. Her hereditary and social hygienic backgrounds were uninformative.

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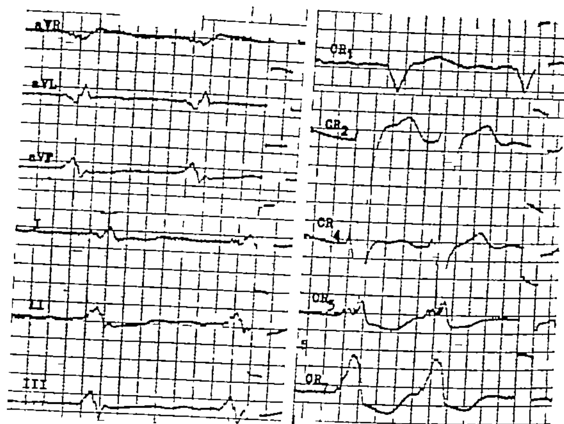


Fig 1 Electrocardiographic tracing showing atrial fibrillation, left bundle branch block and abnormal initial deflections in lead aVL, I II CR₄ and CR (delayed Q")

In the spring of 1958 when she was 37 the patient s current disease first began with some attacks of cardiac palpitation, vertigo and fatigue. She fainted at work and was admitted to the local hospital, where the ECG disclosed widened ventricular complexes, regular rhythm, fast rate and absence of P wa es. At subsequent examination her hemoglobin (Hb) concentration fell slowly and the ESR rose gradually

After some months the liver became palpable. The patient now exhibited peripheral cyanosis. The ECG showed a QRS interval of 0 12 sec. and a variable atrioventricular block. X ray of the heart and lungs disclosed a general enlargement with a cardiac volume of 550 ml/m of body surface area and no pulmonary abnormalities. In due course the cardiac volume increased and 6 months after onset of symptoms it was 875 ml/m body surface area. Radiographic signs of pulmonary stasis had now supervened At auscultation over the heart a weak systolic murmur maximal over the apex and an accentuated second sound over the entire cardiac region were heard.

The patient was admitted to Sahlgrenska sjukhuset in June 1959 She then suffered from severe orthopnea but had neither swollen legs nor nycturia She was markedly cyanotic had spoon-shaped nails on fingers and toes and exhibited jugular stasis. The auscultatory heart sounds were as before. Th blood pressure was 120/70 mm Hg The liver was palpable a finger breadth below the costal margin.

Normochromic anemia was present. The number of white blood cells and the dif ferential count were normal. The platelet count was somewhat low The reticulocytes increased from 4 to 11 The serum iron level was normal. Bone marrow smears had a composition similar to that characterizing sideropenic conditions. The antistreptolysin titer (AST) was normal. The C-reacti e protein level (C-RP) was between 2 and 4 units. The electrophoretic serum protein pattern exhibited no significant abnormalities. No LE-cells were discovered in any of three

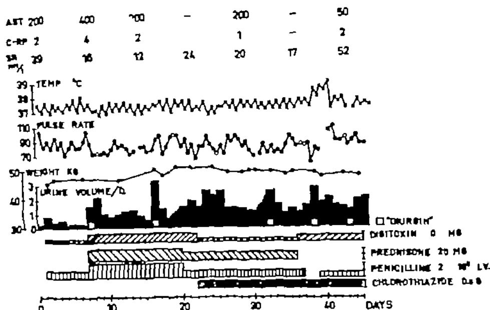


Fig. 2. Clinical course (for explanation see text).

specimens. Liver function tests were normal. The serum electrolytes were normal. Wassermann test was normal, as was Widal's reaction. No cold agglutination could be demonstrated. Paul-Bunnel's reaction was positive in a dilution of 1:16.

While the patient remained in hospital, the ECG exhibited atrial fibrillation with an erratic and irregular ventricular rate (between 85 and 170/min.) and also left bundle branch block with QRS interval of 0.15 secs. In addition ventricular extrasystoles occurred from time to time. On one occasion there was a temporary period of rapid and perfectly regular ventricular rhythm (with rate of 105 beats/min.) and with aberrant ventricular complexes completely unlike those in previous tracings but resembling those of rapid idioventricular rhythm. All other ECG recordings during the entire observation period showed the typical pattern of left bundle branch block, with widened and notched ventricular complexes and discordant ST and T changes. Abnormal initial deflections of the QRS complex were observed in leads representing the lateral wall and diaphragmatic surface of the left ventricle. According to Cap-man and Pearce (2) and our observations (9) these changes (delayed Q) were interpreted as signs of local myocardial damage (fig. 1).

Spirometric examination of the lungs disclosed no signs of obstructive or restrictive ventilatory alterations. Cardiac output, as determined by the dye dilution method (13) was as low as 2 l/min.; with a stroke volume of 20 ml. A simultaneous determination of the oxygen consumption was 265 ml/min. the arterial oxygen saturation was within normal limits (95.6 %) and the oxygen capacity amounted to 13 % by volume.

The heart and lungs were X-rayed repeatedly over a period of two months. The heart was enlarged and had a relative volume ranging from about 900 to 1,000 ml/m² body surface area. The left ventricle seemed to be markedly enlarged probably due to dilatation. No calcifications were observed in the valves, pericardium or myocardium. The thoracic aorta exhibited no manifest abnormalities. Moderate pulmonary stasis was present.

An electrokymographic examination disclosed signs of a fairly extensive but localized myocardial lesion in the lower posterior portion of the left ventricle, with completely paradoxical ventricular movements during



Fig 3 Heart opened to show left atrium and chamber with mitral valve Note diffuse whitish thickening of mural endocardium and slight aneurysmal bulge (arrow)



Fig 4 Mural endocardium from left ventricle massively thickened by fibroelastous tissue with typical downgrowth into myocardium. × 25 van Gieson.

both systole and diastole, similar to those seen in cardiac aneurysm Moreover the abrupt cessation of the rapid filling phase on the left side with a rapid lateral movement of the atrium during early diastole and the high diastolic plateau during the remainder of diastole suggested that atrial outflow was impeded by excessive diastolic pressure in the left ventricle. The large vessels exhibited a remarkable early diastolic collapse, the changes being of the type sometimes accompanying low stroke volume.

Data concerning the course of the patient's disease and therapeutic measures are given in fig 2. The temperature was subfebrile with irregular fluctuations. The highest temperatures were recorded from the 3rd to the 5th day after withdrawal of prednisone and also from the 2nd to the 4th day after withdrawal of penicillin. During the period of prednisone and penicillin medication the C-RP and AST titers diminished gradually from peaks of 4 and 400 respectively attained immediately before the commencement of prednisone therapy The ESR was lower on average during than before or after the treatment with prednisone and penicillin. The leukocyte count, as well as the Hb concentration, remained essentially unchanged. The cardiac insufficiency was treated symptomatically with digitoxin and mercurial diuretics as well as chlorothiazide. Initially during the course of prednisone, the patient showed a tendency to a slight increase in weight. This could be checked temporarily by giving occasional doses of mercurial diuretics. A gradual weight loss followed institution of daily chlorothiazide treatment combined with mercurial diuretics at regular intervals. Although the clinical picture varied a good deal from one day to another the cardiac insufficiency seemed to improve somewhat during the latter half of the hospitalization period. On the 10th day after withdrawal of prednisone, that is on the 5th day after the last febrile period with a peak temperature of 39° C, the patient suddenly died.

Autopsy findings

The heart weighed 480 g and showed dilatation particularly of the left half. Whilst moderate myocardial hypertrophy was manifest in the right auricle and ventricle appearances in the left ventricle were difficult to evalute owing to the conspicuous endocardial lesion. The mural endocard um of the left auricle and ventricle exhibited extensive massive th ckening and had a milky white colour The lesion attained a thickness of several millimeters in the lateral, dorsal portion of the left ventricle where in addition, the underlying myocardium was abnormally thin so that this part of th ventricle formed a suggested aneurysmal bulge (fig 3) In the

right auricle too the endocardium was diffusely though less markedly thickened. The right ventricle exhibited irregular patches of the same whitish thickening. All ostia were free from abnormalities. The valves were thin and slender. No gross signs of endocarditis were present. The myocardium was light brown apart from occasional, minimal streaks in slightly greyer tone. The coronary vessels were of normal caliber and had smooth intima.

The lungs displayed advanced, predominantly chronic stasis. Both lungs, particularly the inferior lobes, were the site of several, up to walnut-sized, old infarctions. Both kidneys exhibited couple of scars after old infarctions. A large number of small submucous vesicles and several small punched-out erosions were observed around the cervical os and in the vagina. Other organs were affected by severe chronic stasis.

At macroscopical examination numerous sections from all chambers of the heart were found to exhibit the typical picture of endocardial fibroelastosis with hyaline and fibrotic thickening containing dense streaks of elastic fibrils (fig. 4). Here and there irregular cords of fibrous tissue broadly penetrated the underlying myocardium, and on its boundary towards the endocardial lining atrophic remnants of muscle fibres in connective tissue and one or two small accumulations of fibrotic adipose tissue could often be observed. Sections from both ventricles presented one or two small foci of inflammatory cellular infiltration into the endocardium with intertrabecular adhesions and residues of mural thrombi in the neighbourhood. The invasive cellular elements were mainly lymphocytes and histiocytes with few plasma cells, the condition being interpreted as small foci of chronic endocarditis, possibly induced by detached thrombi. No other signs of endocarditis were encountered. The valves were normal. No vascular lesions were found. Apart from the layer bordering on the endocardium, the myocardium seemed free from degenerative changes, although the perivascular connective tissue may have been increased in some patches. It was free from invasive cells.

At microscopic examination lesions due to stasis were found in other parenchymal organs and old infarcts in lungs and kidneys. The vaginal changes were remarkably similar to those seen in so-called vaginitis emphysematosa, an uncommon condition *per se* without any obvious relationship with the main disease. No abnormalities were encountered in sections of lymph nodes, brain, pituitary or adrenals.

Cardiac blood and tissue samples of endomyocardium, lung and spleen were subjected to bacteriological and virological cultivation. Cardiac blood yielded no bacterial growth. The other tissue cultures exhibited a sparse growth of coliform bacteria and enterococci. Attempts to isolate viruses in tissue culture and in mice aged six weeks were unsuccessful.

Discussion

The clinical picture in this case was one of severe, rapidly progressive cardiac insufficiency without signs of valvular heart disease. This was accompanied by an accelerated ESR, a positive C-RP titer and anemia. The ECG findings varied from examination to examination. Both the ECG and electrokymography suggested the presence of myocardial damage in the lateral wall of the left ventricle. Coronary arteriosclerosis with infarction was deemed extremely unlikely in a woman of 38 without predisposing diseases. Whilst infectious myocarditis or a myocardial affection associated with a systemic disorder — for example panarteritis nodosa, lupus erythematosus disseminatus or primary amyloidosis — might be suspected, there was no convincing evidence that the patient suffered from any such disease. Nor could any manifestations of pericardial disease be demonstrated. To establish a basis for therapy it was decided to settle for a diagnosis of localized (owing to the ECG and electrokymographic findings) myocarditis.

The autopsy findings, gross endocardial thickening in all cavities of the heart and total absence of other signs of cardiovascular disease are wholly compatible



Fig 3 Heart opened to show left atrium and chamber with mitral valve. Note diffuse, whitish thickening of mural endocardium and slight aneurysmal bulge (arrow)



Fig 4 Mural endocardium from left ventricle, massively thickened by fibroelastosis tissue, with typical downgrowth into myocardium. × 25 van Gieson.

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at the same time as the C-RP and AST titers rose.

Prednisone in moderate doses seems to have been effective, both on the general condition and on the C-RP and AST titers. Death took place after a febrile reaction with accelerated ESR and increased C-RP following prednisone withdrawal. Dyson and Decker reported improvement of the patient's condition in one of their cases in which prednisone was administered. The patient could be discharged from the hospital but died at home a few days later. Conceivably a more consistent steroid treatment with larger doses combined with vigorous therapy for cardiac insufficiency would have more beneficial and lasting effects. Care should be taken when this therapy is withdrawn.

Summary

A case of primary endocardial fibroelastosis in an adult is described. The possibility of differential diagnosis and therapy are discussed.

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with the entity of primary or idiopathic endocardial fibroelastosis, and so are the histological features of the endocardial lesion Laterally in the dorsal wall of the left ventricle — the very region where ECG and electrokymograph indicated the presence of localized myocardial damage — the endocardium showed the greatest thickening and the myocardium was so thinned out that this part of the wall formed a suggested aneurysmal bulge. No vascular abnormalities were manifest either in this region or else where in the heart. The only conceivable explanation for the formation of an aneurysm in this region is that the endocardial process there had encroached upon the myocardium in a degree suf ficient to weaken locally the wall of the heart

The occasional small foci of inflammatory cells encountered in the trabecular network of the endocardium in both ven tricles are not typical of endocardial fibroelastosis. However the histological appearances suggest that they constitute reactions to detached mural thrombi. The autopsy disclosed multiple old infarcts in lungs and kidneys.

A reliable diagnosis of endocardial fibroelastosis cannot be made during life (8) Dyson and Decker (4) reviewed 89 cases of the primary as well as the sec ondary type of endocardial fibroelastosis in adults Taking the series as a whole the predominant clinical manifestations were cardiac insufficiency (93 %) cardiac enlargement (90 %) signs of peripheral embolism (43 %) atrial fibrillation (19 %) bundle branch block (17) and eosinophilia (11 %) Men and women were represented in the ratio of 3 5 to 1

As a contribution to hemodynamics the electrokymographic observations made in this case are noteworthy The fast and deep dip during the rapid-filling phase followed by a high plateau during diastasis, on the left side, may simulate hemodynamic findings in constrictive pericarditis. Hemodynamics in these two conditions could well be similar because a thickened endocardium restricts ventricular expansion during diastole (10) The electrokymographic observations in our case also resemble the pressure-curves from the right ventricle and atrium that have been described by Shillingford and Somers (11) in cases of secondary endocardial fibroelastosis, where the endocardial lesions were very extensive usually comprising also the right half of the heart With less extensive lesions pressure-curves from the right side of the heart showed nothing abnormal. In cases where fibroelastosis is limited to the left half of the heart catheterization of the left side will be necessary to show abnormalities in pressure-curves. Theoretically the same hemodynamic observations ought to appear also in cases of extensive myocardial fibrosis, because of changes in compliance.

Accordingly electrokymographic examination and/or heart catheterization are indicated in all cases of cardiac insufficiency of obscure origin In such obscure cases positive hemodynamic findings, as described above combined with clinical manifestations satisfying Dyson and Decker s criteria might constitute a basis for an intravital diagnosis of endocardial fibroelastosis.

With respect to the therapeutic problem it is remarkable how poorly this patient responded to routine treatment for cardiac insufficiency The pulse rate showed practically no response, not even after large doses of digitoxin.

It is hard to evaluate the effect of a 6-day penicillin treatment. The ESR fell

at the same time as the C-RP and AST titers rose.

Prednisone in moderate doses seems to have been effective, both on the general condition and on the C-RP and AST titers. Death took place after a febrile reaction with accelerated ESR and increased C-RP following prednisone withdrawal. Dyson and Decker reported improvement of the patient's condition in one of their cases in which prednisone was administered. The patient could be discharged from the hospital but died at home a few days later. Conceivably a more consistent steroid treatment with larger doses combined with vigorous therapy for cardiac insufficiency would have more beneficial and lasting effects. Care should be taken when this therapy is withdrawn.

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Haemorrhagic Diathesis, Fibrinolysis and Fibrinogenopenia in Prostatic Cancer

Report of a Case

By

Helge Skjetad and Jon Lamvik

Jürgens and Trautwein (15) in 1930 were the first to report a haemorrhagic diathesis resulting from idiopathic fibrinogenopenia in a patient with prostatic cancer Tagnon et al. (22) in 1952 described the first verified cases of fibrinogenopenia with fibrinolysis in such patients. They were able to extract a proteolytic enzyme from normal and from cancerous prostatic tissue and also from the metastases. They found that this enzyme proteolyzes fibrin and fibrinogen as well as other clotting factors (23) They assume that the proteolytic enzyme digests the fibrinogen in the blood thereby causing the low fibrinogen level. Another possibility is that intravascular coagulation initially consumes fibrinogen and other clotting factors and that fibrinolysis is a secondary phenomenon (18)

Several substances have been found to be inhibitors of fibrinolysis. In 1957 workers in Japan (16) found that epsilon amino-caproic acid (EACA) is effective in the treatment of pathological fibrinolysis. EACA has been shown to block the activation of plasminogen and it is also an antiplasmin (1 5)

We have recently observed a case of prostatic cancer with an extensive fibrinolytic activity and probable fibrinogenolysis causing fibrinogenopenia. A preliminary report of this case has been given earlier (21) In this article we report both the clinical course and the post-mortem examination, which may throw light on certain problems in connection with malignant diseases and fibrinolysis.

Methods

The blood was collected and centrifuged and plasma stored as described by Blix (7) The plasma clot-lysis time was measured as described by the same author Fibrinolytic activity was also determined on unheated fibrin plates by the method of Astrup and

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Müllertz (4). All fibrin plates with test material were incubated for 20 hours at 37 C. The product of two perpendicular diameters of the lysed area was taken as the measure of fibrinolytic activity. All examinations were performed in triplicate. Fibrinogen was determined as fibrin by the method of von Porath (17) and Goa (11). The Schneider test (19) with the modification given by Hjort (13) was also applied. Thrombin coagulation time was determined by a method described by Scott (20).

Case history

The patient was a 74-year-old man who had had dysuria during the last two years. In June 1960 his prostate was found to be enlarged, hard and knotty. X-ray examination revealed widespread metastatic growth in the skeleton. Acid phosphatase 1.6 Bodansky u. On July 8th 1960 he underwent transurethral resection. Histological examination of the prostate showed adenocarcinoma. After the operation he was treated regularly with diethylstilbestrol at a dose of 5 mg daily. In the beginning of August 1961 he developed a series of haematomas as well as haemoptysis and melaena.

On admission to the hospital on Aug. 10th 1961 he had haemoptysis, haematuria and melaena. The clinical examination revealed several large haematomas and numerous petechiae on different parts of the body and bleeding in the mouth. He had gynaecomastia. B. P. 180/90. Hb 86 %. W. B. C. 8,100/mm. blood platelets 107 000/mm. ESR 3 mm/h. There was no coagulation of the blood after 20 min. After the addition of two parts of normal plasma to one part of the patient's plasma a clot formed which dissolved after 18 hours. The prothrombin-proconvertin value (Owren) was 137. The Schneider test 1/25, which corresponds to a fibrinogen concentration of between 25 and 40 mg/100 ml. The fibrinogen level was less than 40 mg/100 ml, determined at the Institute for Thrombosis Research, Oslo, by the method of Jacobsson (14) with the modification of Blombäck and Blombäck (8). Bleeding time 3.5 min. Blood urea 63 mg/100 ml. Acid phosphatase 1.2 Bodansky u. Alkaline phosphatase 1.8 Bessey Lowry u. Thymol turbidity 3.4 Maclagan u. His urine contained protein and blood. Specific gravity 1.029. Microscopic examination of the urine revealed numerous erythrocytes.

The patient received 15 mg diethylstilbestrol 3 times daily and 10 mg prednisone 4 times daily. His bleeding symptoms continued, however, and during the first 5 days of his admission the haemoglobin decreased from 86 to 65 (fig. 1). Before he was given blood on August 15th a sample of his blood formed a little clot which rapidly dissolved. Between Aug. 15th and Aug. 22nd the patient was given 3 l blood. His haemorrhagic diathesis continued, however, and the haemoglobin decreased from 65 to 31 %. On Aug. 22nd and 25th he received 1 g human fibrinogen daily and from Aug. 22nd to Aug. 24th 1.5 l blood. During this treatment the haemoglobin increased temporarily.

On Aug. 26th he received 2 doses of 9 g epsilon-amino-caproic acid (EACA) orally. After that time he was apparently moribund and quite unable to take any more orally. Coagulation studies on a sample drawn after EACA had been given showed improvement. The fibrinogen level had increased and the thrombin coagulation time decreased. He then received 2 g EACA intravenously every 3 hours over a 36-hour period. There was a dramatic response. The bleeding stopped promptly. There was a marked improvement in the clinical condition. On Aug. 26th he received 0.5 l blood and the haemoglobin increased from 38 to 53. During treatment with EACA the fibrinolytic activity fell to normal and the fibrinogen level reached normal values. The thrombin coagulation time was also normalized. On Aug. 28th he was able to take EACA orally, and he was given 9 g every 4 hours and later 6 g every 4 hours.

On Sept. 1st there was a significant drop in the whole blood clotting time. A capillary sample clotted immediately. On the same day the patient developed thrombosis in the left leg. Simultaneously a slight decrease in the fibrinogen concentration was demonstrated while the fibrinolytic activity was still normal. On Sept. 4th anticoagulant treatment with Sintrom (3-(α-(4-nitrophenyl)-β-acetyl)-4-oxycumarin) was started. Due to side effects consisting of hypotension and collapse, EACA was withdrawn on September 4th. On with-

Fig. 1. Variations in fibrinolytic activity, plasma fibrinogen values, thrombin coagulation time and haemoglobin. The treatment used is shown on the diagram.

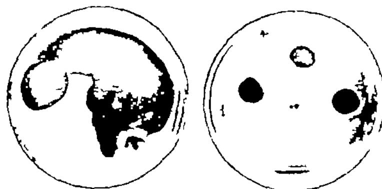


Fig 2. The fibrin plate to the left, dated Sept. 11th 1961 shows extensive fibrinolytic activity with confluence of the three lysed areas. The fibrin plate to the right, from the next day still shows increased fibrinolytic activity but this is considerably reduced during the day of treatment with EACA. The two lower circles on each plate represent normal control plasma.



Fig 3. Microscopical picture of the lung showing the intravascular location of the tumour cells. No thrombosis is to be seen. H E. × 180



Fig 4 Pulmonary vessel containing fresh and degenerating tumou cells and some fibrin strands. P T A. H × 140

drawal of the drug the fibrinolytic cuvity increased again and on Sept 11th extensive fibrinolysis was recorded. Plasma clot-lysis time decreased to 2 5 hours and a confluence of the lysed areas was demonstrated on the fibrin plate (fig 2) On the same day marked fibrinogenopenia was recorded (fig 1) but no bleeding and no increased coagulability or thrombosis was observed. On Sept. 11th the prothrombin-proconvertin value was 29 %. EACA was readministered in a dose of 3 g 6 times daily and the fibrinolytic activity again decreased. In a sample drawn on Sept. 12th the lysed areas on the fibrin plates still showed increased fibrinolytic activity but the fibrinolysis was reduced considerably during the day of treatment (fig 2) The fibrinogen content again increased. On Sept. 21st the patient received 0.5 l blood and developed increased fibrinolytic activity and a significant decrease in plasma fibrinogen which lasted for 2 days. On Oct. 5th another blood transfusion was followed by a shortening of the plasma clot-lysis time but no fibrinogenopenia occurred W th these two blood transfusions no bleeding was observed, the patient did not feel as sick as after the previous blood transfusions, and an adequate rise in the haemoglobin level followed The prednisone was gradually discontinued. Due to side effects consisting of nausea and dizziness EACA was withdrawn on Oct. 17th. The plasma clot-lysis time was significantly shortened, but normalized after readministration of EACA. From Oct. 29th his condition deteriorated rapidly He developed haematomas on different parts of the body and went into coma and died on Nov 11th. The pat ent received a total dose of 1 170 g of EACA.

Autopsy

On post-mortem examination some subcutaneous haemorrhages were found, but no marked extravasation of blood into the internal organs.

The lungs were heavy (1 100 g) and solid. This was most marked in the basal parts. No

thrombi were noted in the pulmonary vessels, or in the extremities. No gross metastases were found apart from osteosclerotic areas in the spinal column. The meninges over the right cerebral hemisphere were markedly thickened and haemorrhagic. There was blood in the subdural space.

Microscopical examination

An anaplastic adenocarcinoma, infiltrating the capsule, was found in the prostate.

In several sections from all lobes of the lungs groups of hyperchromatic cells (fig. 3) were found in the arteries and capillaries. The cells had the same appearance as those found in the prostate. Most of these groups were composed of separate cells without intercellular connections. In some arterioles a few acinar arrangements were seen. Some cells in mitosis were observed. Cells were found in capillaries of the smallest size, with single cells filling out the lumen of the capillary. No damage of the capillary or arteriolar walls was noticed and no tumour cells were found in the parenchyma outside the vessels.

No thrombotic occlusions of the vessels were found, but in some arteries a few thin fibrin strands were noted. These were seen in vessels containing degenerating tumour cells (fig. 4). In some arteries strands of organized hyaline connective tissue were found, surrounding groups of tumour cells. In the right lower and middle lobes acute pneumonia was evident.

In the liver the normal lobular structure was present. Some groups of tumour cells were found in the sinusoids. Some of the groups seemed to replace the parenchyma, but no infiltrating growth was evident.

In the meninges multiple intravascular tumour emboli were found with surrounding haemorrhage but without extravascular tumour growth.

In the spinal column osteosclerotic metastases were present, but in some areas the tumour cells were mainly in the capillaries and sinusoids.

Discussion

The reported case had cancer of the prostate and widespread metastatic growth in the skeleton. The patient was treated with diethylstilboestrol for 1 year after prostatectomy. He then developed a haemorrhagic diathesis. On admission to hospital he had increased fibrinolytic activity and fibrinogenopenia. He was treated with large doses of diethylstilboestrol and prednisone, but without success. His condition deteriorated during repeated blood transfusions, which probably led to an increase in the fibrinolytic activity. Blix (7) has demonstrated increased fibrinolytic activity after blood transfusions, probably caused by leukocyte agglutination, in a patient with a giant haemangioma, thrombocytopenia, fibrinogenopenia and fibrinolytic activity. When the present patient was apparently moribund, therapy with EACA was instituted and gave a dramatic response. The fibrinolytic activity decreased, the plasma fibrinogen level increased and the tendency to haemorrhage disappeared. Further blood transfusions led to increased fibrinolytic activity and once to a transient decrease in fibrinogen but an adequate rise in the haemoglobin level. After these transfusions the patient did not feel sick as after the previous blood transfusions.

Near-fatal haemorrhage may be the result of fibrinogenopenia. It may however also be due to lysis of fibrin which is formed during normal haemostasis.

The intermittent treatment of this patient with EACA in various doses partly determines the form of the curves showing the fibrinolytic activity. There is a relationship between the degree of fibrinolytic activity and the concentration of fibrinogen. The fibrinogen curve reflects the curve showing the fibrinolytic effect on fibrin plates. This suggests that fibrinogenolysis may have been the cause of the fibrinogenopenia in our patient.

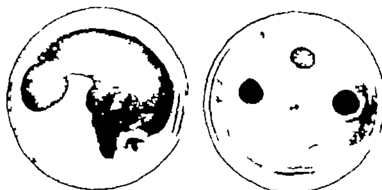


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anticoagulants or fibrinolytic agents in order to prevent metastases.

The microscopical findings in our case and similar findings in four cases of metastatic carcinoma reported by Frick (10) suggest that increased fibrinolytic activity may decrease the ability of tumours to form parenchymatous metastases in human beings also. In all the patients with increased fibrinolytic activity solid metastases were found in the bones, while none showed gross pulmonary or liver metastases, although multiple intravascular tumour emboli were found in the lungs. No thrombus formation was observed in the pulmonary capillaries, apart from occasional fibrin strands in one of the patients reported by Frick and in our case. In view of the sequence of events found in experimental metastatic carcinoma (9, 12) it is reasonable to suppose that the lack of penetration of the vascular walls is due to the fibrinolytic activity.

Summary

A case of nearly fatal haemorrhage is reported in a patient with metastases from a prostatic cancer operated on one year previously. He was treated with large doses of diethylstilboestrol and prednisone but without success. The condition deteriorated during repeated blood transfusions, which probably only led to increased fibrinolytic activity. When the patient was apparently moribund, therapy with EACA (epsilon-amino-caproic acid) was instituted, and gave dramatic response. The fibrinolytic activity decreased, the plasma fibrinogen level increased, the tendency to haemorrhage disappeared, and further blood transfusions led to an adequate rise in the haemoglobin level. Later on the fibrinolytic activity varied in relation to the EACA treatment, suggesting that fibrinogenolysis was the main reason for the haemorrhagic tendency in this patient.

Post mortem examination revealed multiple intravascular tumour emboli in the lungs, but no thrombus formation was observed in the pulmonary capillaries. No damage to the capillary or arteriolar walls was noticed and no tumour cells were found in the parenchyma of the lungs outside the vessels. It is reasonable to suppose that this lack of penetration of the vascular wall is due to the fibrinolytic activity. Our observations suggest that fibrinolytic agents may be of use in the treatment of patients with carcinoma in order to prevent metastases. Combined treatment with fibrinolytic and cytotoxic agents may perhaps prove best.

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During anticoagulant treatment the platelet count did not rise. This observation suggests that intravascular coagulation was probably not the cause of the thrombocytopenia and the fibrinogenopenia in our patient (6) From Sept 4th the patient was given anticoagulant therapy and two days later increased fibrinolytic activity was recorded On Sept 11th the adequate anticoagulant treatment together with the pathologic fibrinolysis should have been adequate to protect the patient against intravascular coagulation Therefore it is more likely that fibrinogenolysis was the cause of the marked fibrinogenopenia on the day mentioned

The most remarkable finding at the post mortem examination was the mainly intra-arterial and intra-capillary location of the tumour metastases. We have not previously found this feature, to such a marked degree in autopsies performed on patients with malignant tumours. We believe that this phenomenon is in some way related to the marked fibrinolytic activity which was evident during the last few months of the patient's life

Several animal experiments, reported in the medical literature, explain this relationship

Warren and Gates (24) showed by the intravenous injection of Walker carcinoma 256 cells into white rats that hyaline thrombi appeared early around the tumour cells that lodged in the pulmonary arterioles and capillaries. Penetration of the vascular wall occurred subsequently A similar phenomenon was observed (5) in mice showing spontaneous metastases from a subcutaneously transplanted tumour Wood (25) in experiments with a vascularized chamber in the rabbit ear found the following events to occur after injection of V_2 carcinoma cells into the auricular artery Injected cancer cells passed into the capillary bed where they became firmly adherent to the endothelium. After a few minutes, intracapillary thrombus formation was noted around the tumour cells. Endothelial damage was then noticed, and leukocytes were found emigrating through the capillary walls, followed by cancer cells. Tumour cells were found to reach the perivascular connective tissue within 3—6 hours, and progressive tumour growth followed

The ability of the tumour cells to establish metastases in these experiments seemed to be related to the ability to attach themselves securely to the endothelium and to the formation of a surrounding thrombus.

Carcinoma cells may show thromboplastic activity as in pancreatic carcinoma, with a marked tendency to intravascular clot formation. Other carcinomas, such as prostatic carcinomas, may also show fibrinolytic activity This activity may counteract the tendency to intravascular clot formation.

The influence of fibrinolytic enzymes on the ability to set up metastases has been shown in animals. Fibrinolysin inhibited the formation of pulmonary metastases when the V_2 carcinoma was inoculated into rabbits (9) Fibrinolysin also resulted in a diminution in pulmonary (12) and hepatic (2) metastases in experiments with the Walker carcinoma 256 in rats, and in the number of hepatic metastases after intravenous inoculation with the Brown Pearce carcinoma in rabbits (9)

Thus, inhibition of the coagulation mechanism may also inhibit metastatic growths in the lungs and other organs. As far as we know human patients with malignant tumours have not been given

anticoagulants or fibrinolytic agents in order to prevent metastases.

The microscopical findings in our case and similar findings in four cases of metastatic carcinoma reported by Frick (10) suggest that increased fibrinolytic activity may decrease the ability of tumours to form parenchymatous metastases in human beings (10). In all the patients with increased fibrinolytic activity solid metastases were found in the bones, while none showed gross pulmonary or liver metastases, although multiple intravascular tumour emboli were found in the lungs. No thrombus formation was observed in the pulmonary capillaries, apart from occasional fibrin strands in one of the patients reported by Frick and in our case. In view of the sequence of events found in experimental metastatic carcinoma (9, 12) it is reasonable to suppose that the lack of penetration of the vascular walls is due to the fibrinolytic activity.

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On the Variation of the Time of Onset and of Death of Myocardial Infarction

By

Henning Lindholm

The cause of myocardial infarction is known, but despite considerable investigation our knowledge of the factors deciding the time of the onset of the condition is still meagre.

Thus, in recent years various articles have appeared suggesting that certain *climatic conditions* may precipitate the onset of acute coronary occlusion. On the basis of an analysis of data from official Norwegian statistics (Norska statistiska centralbyrån) for 1951—1955 Jervell (6) found myocardial infarction to be more common in winter than in summer He found deaths from diseases of the coronary arteries to be more common in November December and January than during the summer months, with a minimum frequency in August. But for some unknown reason the mortality among the males showed an extra peak in July In a series of 132 cases of myocardial infarction with known date of onset in 105 Jensen (5) found that most cases occurred in December and May In Dotzauer and Naeve's (3) series of 1,013 cases of fatal coronary thrombosis in Hamburg the mortality was substantially higher during the cold winter months. In contrast, Master and Jaffe (9) found only a slight seasonal variation in their series of 2 080 cases of coronary occlusion, the number of attacks during the winter being only 5 % higher than during the summer

No satisfactory explanation can be offered for the increase if any in the frequency of myocardial infarction during the cold winter months. It is believed that the increased frequency of infections of the respiratory tract during this season might be partly responsible, that the increased basal metabolism during the cold winter months places higher demands on the cardiovascular system, and that exposure to the cold elicits vasomotor reflexes. As to the latter assumption, reference is usually made to the work of Kuhn (7) who studied 7 males, aged 20—21 years, and found the electrocardiographic changes after physical exertion to be more marked when the experiment was carried out in the cold than when it was performed at room temperature.

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Some authors have mentioned *changes in the atmospheric pressure and other meteorological conditions* as precipitating factors. Thus, Poumailloux and Viart (11) found myocardial infarction to occur more frequently one or two days after periods of intense geomagnetic activity Nikolaev and Marinow (10) claimed to have demonstrated a distinct correlation between the frequency of myocardial infarction and "unfavourable weather in a series consisting of 113 cases that occurred in Sofia from Jan. 1 1952 to Dec. 12, 1955 Teng and Heyer (12) arrived at a similar conclusion in their investigation of 1 106 cases with a firm diagnosis of myocardial infarction in Dallas in the north of central Texas which is situated 250 miles from the coast The climate there is characterized by hot summers and mild winters with frequent sudden changes in the temperature and moisture of the atmosphere For the period from Jan 1 1946 to Dec. 31 1951 they found the frequency of myocardial infarction to be highest in association with sudden meteorological changes caused by the approach of cold polar air or hot tropical air

As to the tendency of myocardial infarction to occur on *certain days of the week* Berg et al. (1) found a high morbidity on Saturdays and Sundays and particularly on Mondays, while the lowest rate was noted on Thursdays. Jensen (5) found the frequency to be highest on Saturdays and Mondays, Master (8) on Mondays, while Gorbatow et al. (4) found no statistically significant variation with the day of the week and no tendency for the disease to occur during the week-ends.

According to some authors, the risk is greatest during the weekends (Pathologie des Wochenendes) owing to parties or other entertainment on Saturdays a heavy rich meal on Sundays, usually with alcohol, and often very strenuous work on Mondays.

Dotzauer and Naeve (3) found changes in the bloodstream in the very smallest arterioles after ingestion of a fatty test meal During such postprandial hyperlipaemia they also observed a reversible aggregation of red blood cells under the capillary microscope This aggregation was sometimes so marked as to obstruct the blood flow It would thus appear that it is not only a habitual fatty diet that is dangerous, but also occasional fatty meals. According to Berg et al. (1) this so-called sludge phenomenon, i e intravascular aggregation of erythrocytes, can be produced not only by allergic reactions and exposure to the cold but also by fatty meals. Other authors have also found the blood to coagulate quicker after a fatty meal Berg et al. also observed that the prothrombin index was often higher on Mondays than on other days of the week. They ascribed this to the fact that on Sundays most in patients receive visitors and then of course often also some extra dainties.

In his extensive investigation Master (8) found that most cases of myocardial infarction occurred at about 2 o clock in the morning, the next commonest times being 10 o clock and 11 o clock at night. He could not demonstrate any relation between onset and respiratory tract infections or between physical or mental stress and the time of the onset of the attack.

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During the period Feb. 3, 1958 to Feb. 2, 1962, 193 patients (48 females and 145 males) were admitted to Simrishamn hospital because of myocardial infarction. The total number of admissions during this period was 5,471 which means that 1 out of every 28 had myocardial infarction. The youngest male with myocardial infarction was 44 years and the oldest 90. The youngest female with myocardial infarction was 53 and the oldest 84. The number of first attacks was 176 (45 females and 131 males) The overall mortality was 36.8 % (50 % of the females and 32.4 % of the males) The diagnosis was based on the patient's history clinical picture, electrocardiographic findings and the usual laboratory tests, which included transaminase tests when considered necessary Information is unavailable on the month of onset in 1 man, on the day of the week in 4 males and 2 females, and on the hour of the day in 18 males and 15 females. As to the time of death, we have reliable information on the month, day and hour of the day of all. Physical exertion was noted as a possible contributory cause of the attack in 16 males and 3 females. Heavy meals may have favoured the onset in 2 of the males. At the time of the attack 5 males and 3 females had respiratory tract infection. One male and 2 females had bronchial asthma and 1 male had silicosis.

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Table I gives the sex and age distribution and the number of persons who died from their myocardial infarction. As in other series, males were predominant. They represented three-fourths of the material. It is also clear from the table that the age of the females t the time of onset was on the average 10 years higher than that of the males. This explains the higher mortality of the females, i. e. 50 % against 32.4 % of the males. Supposing N to be the total number of cases of myocardial infarction both males and females in a given age-group and D the number of deaths in the same age-group

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Age, years	♂	♀	Dead ♂	Dead ♀
40—49	13	0	1	0
50—59	32	6	7	1
60—69	60	17	17	6
70—79	30	20	17	13
80—89	9	5	4	4
90—99	1	0	1	0
Total	145	48	47	24

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$$\sqrt{\Sigma n_i\, P\ (1-P_i)\, \frac{N_i - n_i}{N_i - 1}}$$

In the present material $\Sigma d_i = 24$ The expected number was 21.2. The difference was 2.8 The mean error of the difference was $\sqrt{10} = 3.2$.

Therefore, as pointed out by Biörck et al. (2) if due consideration be given to age, the mortality will not be found to vary appreciably with sex. In other words, the mortality from myocardial infarction will not be found to vary with sex after elimination of the effect of age.

In table II the cases are distributed according to month of attack and of death.

As to the month of onset, comparison between the observed (0) and expected (E) values according to the formula $X^2 = \Sigma \frac{(0-E)}{E}$ will give $X = 18.09$ Degrees of freedom 11 $P > 0.05$. For the deaths the corresponding values will be $X^2 = 9.96$ Degrees of freedom 11

Some authors have mentioned *changes in the atmospheric pressure and other meteorological conditions* as precipitating factors. Thus, Poumailloux and Viart (11) found myocardial infarction to occur more frequently one or two days after periods of intense geomagnetic activity Nikolaev and Marinow (10) claimed to have demonstrated a distinct correlation between the frequency of myocardial infarction and unfavourable weather in a series consisting of 113 cases that occurred in Sofia from Jan. 1 1952 to Dec. 12 1955 Teng and Heyer (12) arrived at a similar conclusion in their investigation of 1 106 cases with a firm diagnosis of myocardial infarction in Dallas in the north of central Texas, which is situated 250 miles from the coast The climate there is characterized by hot summers and mild winters with frequent sudden changes in the temperature and moisture of the atmosphere. For the period from Jan. 1 1946 to Dec 31 1951 they found the frequency of myocardial infarction to be highest in association with sudden meteorological changes caused by the approach of cold polar air or hot tropical air

As to the tendency of myocardial infarction to occur on *certain days of the week*, Berg et al. (1) found a high morbidity on Saturdays and Sundays and particularly on Mondays, while the lowest rate was noted on Thursdays Jensen (5) found the frequency to be highest on Saturdays and Mondays, Master (8) on Mondays while Gorbatow et al (4) found no statistically significant variation with the day of the week and no tendency for the disease to occur during the week-ends.

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Table IV. The time in day of onset (unknown in 18 males and 15 females) and of death

	Hour of the day								
	0–3	3–6	6–9	9–12	12–15	15–18	18–21	21–24	Total
No. ♂	11	8	20	18	9	20	17	24	127
♀	2	5	4	3	2	4	7	6	33
♂+♀	13	13	24	21	11	24	24	30	160
Dead ♂+♀	7	8	7	7	12	11	4	15	71

number and this number be compared with the observed number of attacks, we get

Males
$X^2 = 7.81$. Degrees of freedom 6. $P > 0.20$.
Females
$X^2 = 2.71$. Degrees of freedom 6. $P > 0.80$.
Males + females
$X^2 = 4.13$. Degrees of freedom 6. $P > 0.50$.

The corresponding values for the deaths were

$X^2 = 3.44$. Degrees of freedom 6. $P > 0.70$.

Thus neither the morbidity nor the mortality was found to vary with the day of the week.

Table IV gives a similar analysis but for the hour of the day. The values obtained for the attacks were

Males
$X^2 = 15.05$. Degrees of freedom 7. $P > 0.02$.
Females
$X^2 = 5.33$. Degrees of freedom 7. $P > 0.70$.
Males + females
$X^2 = 18.40$. Degrees of freedom 7. $P > 0.01$.

The corresponding values for the deaths were

$X^2 = 8.79$. Degrees of freedom 7. $P > 0.20$.

Thus neither the frequency of the attacks nor that of the deaths varied with certainty with the hour of the day. It is possible that the onset tended to occur somewhat less frequently between midnight and 3 a.m. and between 3 a.m. and 6 a.m., but it should be borne in mind that in 15 % of the cases the hour of the onset was not known. Most of these cases might have occurred during the night.

Summary

In an investigation of 193 cases of myocardial infarction (48 females and 145 males) in the south eastern part of Scania (Sweden) no variation was found in frequency of morbidity or mortality with the season of the year, the month of the year, the day of the week or the hour of the day. In the females the onset of the disease occurred 10 years later than in the males, which explains the higher mortality found for the females, it having been shown that the mortality rate does not vary with sex but with age.

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Table II Month of onset (unknown in 1 male) and of death

	Month												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Total
No. ♂ + ♀	17	15	11	10	21	17	7	16	16	20	26	16	192
Expected no. ♂ + ♀	16.3	14.7	16.3	15.8	16.3	15.8	16.3	16.3	15.8	16.3	15.8	16.3	–
Dead ♂ + ♀	6	5	3	6	8	5	1	7	8	5	9	8	71

Table III Day of onset (unknown in 4 males and 2 females) and of death

	Day							
	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.	Sun.	Total
No. ♂	29	21	12	21	17	20	21	141
♀	6	4	9	5	8	7	7	46
♂ + ♀	35	25	21	26	25	27	28	187
Dead ♂ + ♀	14	11	8	10	9	12	7	71

$P > 0.50$ The comparison thus reveals no evidence of any variation in the morbidity or mortality with the month of the year

Similar analysis of the series distributed among the seasons instead of months gave the following results concerning onset.

	Observed no. ♂	Observed no. ♀	Expected no. ♂	Expected no. ♀
Winter (Dec.—Feb.)	36	12	36	12
Spring (March—May)	32	10	36	12
Summer (June—Aug.)	29	11	36	12
Autumn (Sept.—Nov.)	47	15	36	12

Males
$\chi^2 = 5.16$. Degrees of freedom 3. $P > 0.10$

Females
$\chi^2 = 1.16$. Degrees of freedom 3. $P > 0.70$.

Males + females
$\chi^2 = 5.79$ Degrees of freedom 3. $P > 0.10$.

On comparison between this seasonal division and the time of death χ^2 was found to be 2.40 Degrees of freedom 3. $P > 0.30$

The analysis thus showed no seasonal variation of the frequency of the attacks or of the deaths.

In the area served by Simrishamn hospital (Österlen) the weather is very cold and rough in January and February and one might have expected myocardial infarction to be more common during these months than during the rest of the year but judging from the present investigation, this is not the case

In table III the cases are distributed according to day of onset and death. If the expected number for each day of the week be taken as one-seventh of the entire

Table IV The time in day of onset (unknown in 18 males and 15 females) and of death

	Hour of the day								
	0–3	3–6	6–9	9–12	12–15	15–18	18–21	21–24	Total
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Coronary Mortality in Relation to Total Mortality

By

Gunnar Blomqvist and Gunnar Biörck

Official death certificate statistics present a difficult problem to the epidemiologist. With regard to the reliability of death certificate diagnoses, most investigators would probably agree that there are no studies whatsoever that would, by strict criteria, justify the use of such data in scientific work. On the other hand, age-specific mortality rates of various populations are the only quantitative epidemiological data that are easily accessible and often the only available for total populations. They have thus been used extensively particularly in studies on cardiovascular diseases. Many of these investigations have been heavily criticized but it now appears that, at least for coronary heart disease, the large differences between various countries found in official mortality statistics will largely be substantiated by the results from direct population morbidity studies. Therefore, it still may be worthwhile to pay some attention to a problem which becomes apparent as soon as one tries to correlate data on coronary mortality from different countries to factors such as the standard of living, amount of physical exercise, and dietary pattern: the huge differences in coronary mortality among Northwestern European countries which do not seem to differ much with regard to the mode of life.

It is surprising to find that whereas much has been written about these coronary mortality differences, discrepancies with regard to total mortality have been much less discussed. Table I gives coronary (group B-26 according to the International List (1)) and total mortality for the group of countries of primary interest (England, Holland, and Scandinavia) and for a few other countries selected arbitrarily for the sake of illustration. Among the Northwestern European countries the total mortality rate (per 100,000 population) in the age group 55—59 varies from Sweden's 1 060 to Finland's 2,060 and the coronary mortality rate from Holland's 284 to Finland's 639 thus by a factor of 2. Corresponding figures are found in age group 60—64. It is immediately apparent from table 1 that the variation in coronary death rate in this group and age range very closely follows that of the total death rate. In

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Coronary Mortality in Relation to Total Mortality

By

Gunnar Blomqvist and Gunnar Biörck

Official death certificate statistics present a difficult problem to the epidemiologist. With regard to the reliability of death certificate diagnoses, most investigators would probably agree that there are no studies whatsoever that would, by strict criteria, justify the use of such data in scientific work. On the other hand, age-specific mortality rates of various populations are the only quantitative epidemiological data that are easily accessible and often the only available for total populations. They have thus been used extensively particularly in studies on cardiovascular diseases. Many of these investigations have been heavily criticized, but it now appears that, at least for coronary heart disease, the large differences between various countries found in official mortality statistics will largely be substantiated by the results from direct population morbidity studies. Therefore, it still may be worthwhile to pay some attention to a problem which becomes apparent as soon as one tries to correlate data on coronary mortality from different countries to factors such as the standard of living, amount of physical exercise, and dietary pattern the huge differences in coronary mortality among Northwestern European countries which do not seem to differ much with regard to the mode of life.

It is surprising to find that whereas much has been written about these coronary mortality differences, discrepancies with regard to total mortality have been much less discussed. Table I gives coronary (group B-26 according to the International List (1)) and total mortality for the group of countries of primary interest (England Holland and Scandinavia) and for a few other countries selected arbitrarily for the sake of illustration. Among the Northwestern European countries the total mortality rate (per 100,000 population) in the age group 55—59 varies from Sweden's 1 060 to Finland's 2,060, and the coronary mortality rate from Holland's 284 to Finland's 639 thus by a factor of 2. Corresponding figures are found in age group 60—64 It is immediately apparent from table I that the variation in coronary death rate in this group and age range very closely follows that of the total death rate. In

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It is surprising to find that whereas much has been written about these coronary mortality differences, discrepancies with regard to total mortality have been much less discussed. Table I gives coronary (group B-26 according to the International List (1)) and total mortality for the group of countries of primary interest (England, Holland, and Scandinavia) and for a few other countries selected arbitrarily for the sake of illustration. Among the Northwestern European countries the total mortality rate (per 100 000 population) in the age group 55—59 varies from Sweden's 1,060 to Finland's 2,060, and the coronary mortality rate from Holland's 284 to Finland's 639 thus by a factor of 2. Corresponding figures are found in age group 60—64 It is immediately apparent from table I that the variation in coronary death rate in this group and age range very closely follows that of the total death rate. In

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Table I Total and coronary (B 26) mortality in middle aged men, 1958 Per 100,000 population

Country	Age			
	55—59		60—64	
	Total	B-26	Total	B-26
Japan	1,680	114	2,630	187
Portugal	1 480	142	2,350	308
Italy	1 440	266	2,200	460
Holland	1 170	284	1,860	476
England and Wales	1 740	497	2,770	774
Sweden	1,060	312	1,810	568
Denmark	1,210	356	1,950	603
Norway	1 130	356	1,830	558
Finland	2,060	639	3,280	1,030
Canada	1,580	656	2,440	1 016
USA (whites)	1 770	742	2,740	1 157

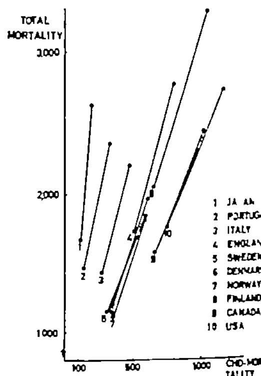


Fig 1 Coronary and total death rates per 100,00[0] population, men 55—59 and 60—64 years of a[ge] 1958 Coronary deaths = Group B-26 accordi[ng] to the international classification.

other words, the variation in coronary mortality expressed as per cent of total mortality is much smaller than the variation in absolute figures. This can also be seen from fig 1 where the figures from table I are presented graphically The plots from the countries of the study group seem to be centered around the same regression line with very little scatter To the left are found examples of countries with a low relative and absolute coronary mortality to the right two countries, USA and Canada, with a high absolute and relative frequency of coronary deaths.

Fig 2 gives the reason why ages 55—59 and 60—64 have been chosen for comparison. Both coronary and total mortality increase with age. The percentage of coronary deaths shows a sharp rise from close to zero in ages below 30 to a value which varies from country to country but in most populations seems to be fairly constant beyond age 50—55 This does not mean that figures concerning ages below 55 are uninteresting but in many previous studies too much emphasis has been put on the coronary mortality of the youn[ger] age groups, and the vast majority of a[ll] coronary deaths occur in ages over 55 A point of some interest is, furthermore that if mortality figures are correlated with morbidity they may be more closel[y] related to the morbidity of the precedin[g] five-year age group than to the morbidity within the same age group depending o[n] the mean survival time after onset o[f] clinical disease.

It seems obvious that there is a cor relation between total and coronary mor tality within this group of Northwestern European countries, but it is not obviou[s] why such a correlation should exist It is very unlikely that the over all mortality differences should be secondary to the dif ferences in coronary mortality as the percentage of coronary deaths does not

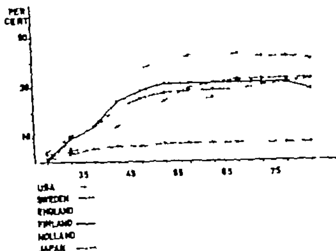


Fig. 2. Coronary mortality (B-26) as per cent of total mortality by age, men, 1958.

vary The percentage of deaths from malignant diseases is also relatively stable within the group. (Holland has a slightly higher percentage of deaths from malignancies than the rest of the group, which fits with its slightly lower percentage of coronary deaths. This relatively high cancer rate has been related to the high Dutch degree of urbanization.) As no satisfactory explanation is readily at hand, it is rather tempting to ask whether the differences in coronary mortality within the group are due to some general mortality factor or factors, differing from country to country whereas one or more factors, common to these countries, determine the relative frequency of coronary deaths.

This view can be further illustrated by a comparison between Finland and Sweden. As appears from the table, the Finnish figures for coronary and total mortality are about twice as high as the Swedish. It can also be seen in fig 2 that the curve for the relative frequency of coronary deaths by age in Finland very closely resembles the Swedish curve in shape, but that there is a time lag of about 6 or 7 years. In fig 3 are plotted Finnish and Swedish age-specific mortality rates in a semi logarithmic system. The left part of the figure shows the actual rates. In the right part of the graph, the Swedish curves have been shifted to the right, e.g 55-year-old Finnish men are compared to Swedish men about 62 years of age. It is then found that not only do total death rates agree very closely but also the distribution for various causes of death is very similar Hardin B. Jones (2) has expressed such differences with regard to total age specific death rates as differences in physiological age. According to his terminology Swedes should be 6—7 years younger biologically than Finns of the same chronological age. The parallel Finnish and Swedish curves in fig 2 also fit his view very nicely but it should be pointed out that the rest of the curves in this graph do not show the same degree of similarity

The reasoning implying that coronary mortality in these countries is a function of the over-all mortality can be tested and has in fact already been partly in

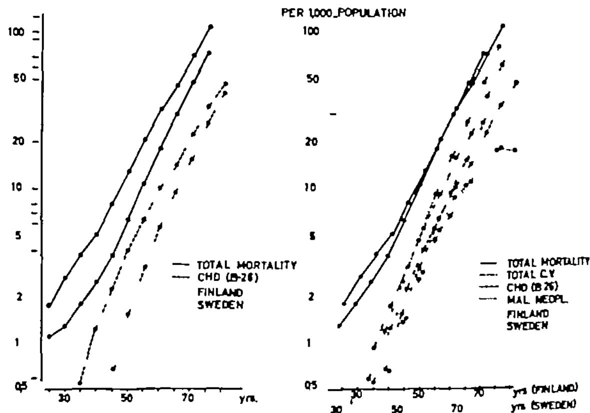


Fig 3. Comparison between Finnish and Swedish male age-specific death rates 1958. The right part of the graph demonstrates the degree of agreement achieved when the Swedish death rate curves have been moved 6.5 years to the left, e. g rates for 30-year-old Finns are plotted against rates for 36.5-year old Swedes, etc.

vestigated by Jones. This was done by calculating abstract death rates [1] e. g by forming an abstract population of the number of deaths from a certain disease grouped by age at death (the number of individuals who are alive in the population for example at 40 years of age are those who died at ages of 40 and older and so on for all ages) and then ordinary life table methods are used in calculating age-specific death rates. These abstract death rates may then be compared to the over all death rates of the population and will thus give an estimate of the "force of mortality of any particular disease as compared to the total mortality Jones found that as far as the countries in the group studied are concerned coronary heart disease seems to be an "average cause of death distinctly different from e g tuberculosis, cirrhosis, and cancer These diseases have a much greater age-specific intensity of the death rate process, whereas the coronary abstract death rates are nearly the same as the age-specific death rates from all causes. Jones based his findings mainly on figures from 1950—53 We have carried out similar calculations of abstract death rates for coronary heart disease in Sweden based on figures from 1958, and have arrived at the same result, viz. that coronary heart disease appears to be an average" cause of death

Population studies on coronary heart disease in the Northwestern European

[1] For a description of the method, see Jones (2)

countries will probably soon be available. These may give the answer to some of the questions that may be raised by the findings discussed above. It will be extremely interesting to make a comparison of the prevalence and incidence of coronary heart disease in similar populations of these areas and also, of course, of the long-term prognosis of comparable groups of patients. Are prevalence and incidence differences among these countries less pronounced than the death rate differences because they are counteracted by a smaller force of mortality operating upon patients with coronary disease? Are the apparent differences in biological age with regard to mortality to be found also in age-specific incidence figures?

Summary

There are great differences in coronary mortality among the countries in Northwestern Europe. These differences are found to be closely related to differences with regard to mortality from all causes. The implications of this finding are briefly discussed.

Acknowledgment

This study was supported by grant from Folksam, which is gratefully acknowledged.

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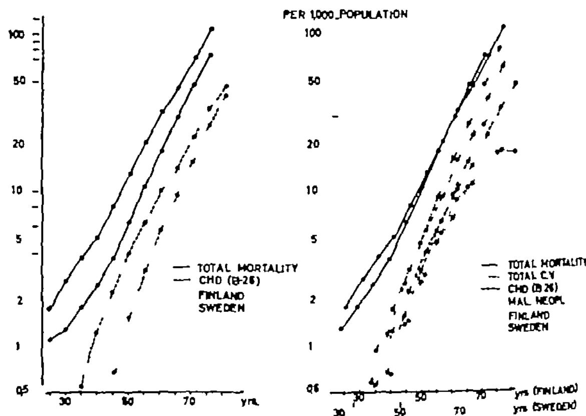


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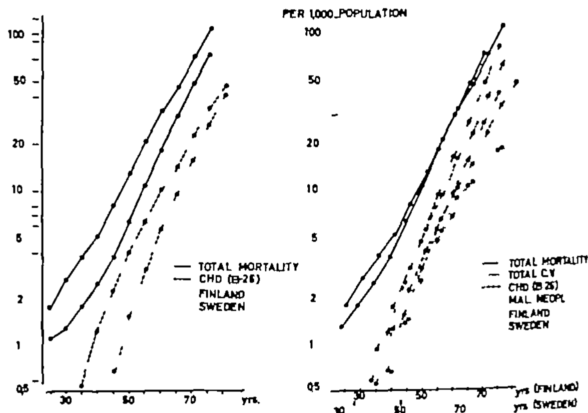


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The Effect of Plasma and Cohn's Fraction I on the Duke and Ivy Bleeding Times in von Willebrand's Disease

By

Christian F. Borchgrevink, Olav Egeberg, Hans Chr. Godal and Peter F. Hjort

Von Willebrand's disease is defined in this paper as an inherited autosomal dominant hemorrhagic diathesis characterized by a prolonged bleeding time and a deficiency of factor VIII (38). The terminology and the relationship to the capillary disorders and to the qualitative platelet deficiencies are not considered here.

In 1956 Nilsson et al. (36) observed that an intravenous infusion of a purified Cohn's plasma fraction I not only increased the concentration of factor VIII but also *normalized the bleeding time.* This observation was extended in subsequent studies (33, 34, 35, 37, 38) leading to the conclusion that the prolonged bleeding time in von Willebrand's disease is caused by the lack of a normal plasma factor.

We were unable to confirm these results with the techniques used in our laboratory. One explanation could be a difference in the bleeding time technique: the Swedish workers used the Duke (18) while we were using the Ivy (26) technique. Therefore, we repeated our studies using both techniques. We found that infusions of plasma or plasma fraction I did shorten the Duke bleeding time, but had little or no effect on the Ivy bleeding time. These results are presented and discussed in this paper.

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Case reports

Case 1. A 13-year-old boy. The maternal grandfather was a bleeder; the mother and five of her siblings had a bleeding tendency similar to that of the patient. From the age of four, he had bruised easily and bled unusually long from minor wounds. During the last two years, he had frequent nose bleedings and was once hospitalized for this reason. He had never had petechiae.

The physical examination was normal at the time of this study.

H. received 500 ml of plasma fraction I, prepared from the plasma of six donors and containing 59 % factor VIII. Two days later 350 ml of blood was withdrawn. He then received 785 ml plasma prepared from the blood of three donors and containing 14,000 platelets/mm³. The plasma was infused over 80 min. with no reaction.

Table I. Hemostatic tests in the patients before transfusions*

Test	Patients no.					Normal	References
	1	2	3	4	5		
Bleeding time, Ivy (min)	> 30	> 30	> 30	> 30	> 30	3—11	(11)
Bleeding time, Duke (min)	> 30	> 30	> 30	> 30	—	1—5	(18)
Tourniquet test (petechiae)	30	35—40	30	40	30—40	<5—10	(45) 90 mm Hg for 5 min
Platelets (1,000/mm³)	458	223	202	184	238	138—421	(39) as modified by (21)
Adhesive platelets in vitro (%)	37	24	38	27	35	26—68	(21)
Adhesive platelets in vivo (%)	—2	—1	5	—1	—1	24—56	(10)
Clot retraction after 3 hrs (cm)	8.0	6.0	7.0	6.8	8.3	5.8—9.3	(48)
Platelet factor 3	Normal	Normal	Normal	Normal	Normal	—	(24)
Whole blood clotting time (min)	3.5	3.5	3	3.5	4	2—5	(23)
Fibrinogen (mg%)	290	302	330	315	278	150—400	(27) as modified by (18)
Cephalin time (sec)	86.5	134	121	68	125	57—64	(19)
Quick time (sec)	15.3	14.8	14.8	15.1	14.7	13—16	Human brain thromboplastin
P & P test (%)	102	88	105	93	83	80—120	(41)
Factor II & X (%)	90	60	92	118	96	80—120	(22)
Factor V (%)	105	150	74	66	60	80—120	(40)
Factor VII (%)	100	110	103	100	70	80—120	(41)
Factor VIII (%)	7	2	7	45	7	60—150	(19)
Factor IX (%)	102	118	70	79	80	70—140	(19)
Factor XI (= P.T.A.) (%)	89	134	87	88	78	70—140	(19)
Fibrinolysis	No	No	No	No	No	—	(4)
Hematocrit (vol. %)	45	40	37	44	41	38—54	(49)

Collection of blood for testing. Venepuncture was performed with a siliconized needle, and the first 2—3 ml of blood were discarded. Nine volumes of blood were then collected directly into a plastic tube containing one volume of 3.13 % w/v sodium citrate dihydrate. This was mixed by gentle inversion and centrifuged at 2,500 r. p. m. (ca. 1,800 G) for 30 min. at 4° C. The plasma was removed with a siliconized pipette and stored in plastic tubes at —20° C. until assayed.

Collection of blood from donors. Fasting, compatible blood donors were bled by means of the Fenwal equipment (Fenwal Laboratories Inc., Framingham, Mass.) 500 ml of blood was collected through a siliconized needle and plastic tubing into a plastic bag containing 75 ml acid-citrate-dextrose solution, U. S. P. formula A. The blood was discarded if there were any difficulties during collection.

Preparation of plasma for infusion. Immediately after collection, the blood bags were centrifuged at 2,500 r. p. m. (ca. 1 400 G) for 30 min. at 4° C. The clear platelet-poor plasma was then squeezed into a plastic bag

Case 2 An 18-year-old female. A maternal great-grandfather was probably a bleeder and the patient's sister bled to death as an infant. From the age of 15 months, the patient had bled abnormally easy bruising prolonged bleeding from small wounds and during dentition joint bleeding on two occasions (knee and ankle) and profuse menstrual bleeding since menarche at the age of 15 years, but she had never had petechiae. She had been hospitalized 25 times and had received many transfusions. She had constantly been taking large doses of iron, because of iron-deficiency anemia.

The physical examination gave normal results at the time of this study

She received 500 ml of plasma fraction I prepared from the blood of six donors and containing 61 factor VIII. Two days later 705 ml plasma was infused over 90 min. with no reaction The plasma was prepared from the blood of three donors and contained 11 000 platelets/mm³

Case 3 A 41 year-old housewife. There was no bleeding tendency in her family She had a severe bleeding tendency from the age of two years, with easy bruising profuse nose bleeding and heavy menstrual bleeding but no joint bleeding During shorter periods, she had petechiae. She had been hospitalized as an emergency case many times and had received many transfusions. For a year she was treated with testosterone with some success, but was finally given X-ray treatment to stop menstrual bleeding

She had a moderate iron-deficiency anemia and a slightly enlarged spleen otherwise the physical examination gave normal results at the time of this study

A plasma transfusion of 760 ml was given over 120 min. She had a slight chill after 45 min. The plasma was prepared from the blood of three donors and contained 9 000 platelets/mm³ Three days later she was given 500 ml of plasma fract on I prepared from four donors, containing 87 factor VIII

Case 4 A 44-year-old housewife. Her father his brother a paternal first cousin, three siblings, a nephew and her daughter had a bleeding tendency similar to that of the patient. She had bled abnormally since infancy easy bruising, increased bleedin from small wounds and at dentition (th sockets had to be sutured) excessive menstru bleeding and profuse bleeding after child birth. She was finally castrated by X-ra treatment

The physical examination was normal a the time of this study

She received 1 135 ml plasma over 120 mi with no reaction. The plasma was prepare from five donors and contained 12,00 platelets/mm³ One day later she was give 500 ml plasma fraction I prepared from fou donors, containing 82 % factor VIII

Case 5 A 19-year-old nurse Two of he mother's siblings had an abnormal bleeding tendency and her brother bled to death at th age of three years. She had bled abnormall since infancy easy bruising prolonged bleeding from small wounds and after tooth extraction Since menarche at 16 years, she had heav menstruations and had been hospitalized fo transfusions six times. She had never ha bleeding into the joints or petechiae.

Physical examination was normal at th time of this study

A transfusion of 1 036 ml plasma, prepare from four donors and containing 31 00 platelets/mm³ was given over 55 min. with no reaction. Six days later she received 450 ml plasma fraction I prepared from si donors and containing 48 factor VIII

Material and methods

Bleeding time (Ivy) The technique of Ivy et al. (26) was used, with one modification instead of making punctures with a mechanical stylet, we made cuts with surgical blades (Gilette surgical blades, shape E) each blade being used for one examination only Three cuts were made on the volar surface of the forearm, 5—6 mm long, 1 mm deep and 2 cm apart The blood was carefully absorbed with the edge of a filter paper every 30 sec. the wound itself was not touched

Bleeding time (Duke) The method of Duke (18) was used one small cut was made with the same sharp blades in the lobe of the ear and the blood was removed as described above.

Table I Haemostatic tests in the patients before transfusions*

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Bleeding time, Duke (min)	> 30	> 30	> 30	> 30	—	1—5	(18)
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Clot retraction after 3 hrs (cm)	8.0	6.0	7.0	6.8	8.3	5.8—9.3	(48)
Platelet factor 3	Normal	Normal	Normal	Normal	Normal	—	(24)
Whole blood clotting time (min)	3.5	3.5	3	3.5	4	2—5	(23)
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Factor IX (%)	102	118	70	79	80	70—140	(19)
Factor XI (=P.T.A.) (%)	89	134	87	88	78	70—140	(19)
Fibrinolysis	No	No	No	No	No	—	(4)
Haematocrit (vol. %)	45	40	37	44	41	38—54	(49)

Collection of blood for testing. Venepuncture was performed with a siliconized needle, and the first 2—3 ml of blood were discarded. Nine volumes of blood were then collected directly into a plastic tube containing one volume of 3.13 % w/v sodium citrate dihydrate. This was mixed by gentle inversion and centrifuged at 2,500 r. p. m. (ca. 1,800 G) for 30 min. at 4° C. The plasma was removed with a siliconized pipette and stored in plastic tubes at —20° C. until assayed.

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Preparation of plasma for infusion. Immediately after collection, the blood bags were centrifuged at 2,500 r. p. m. (ca. 1 400 G) for 30 min. at 4° C. The clear platelet-poor plasma was then squeezed into a plastic bag

Table II The effect of plasma and plasma fraction I on the Duke bleeding time The bleeding time was measured before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Duke bleeding time (min)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20-24 hrs	Before	15 min	4 hrs	20-24 hrs
1	> 30	8	11	28	> 30	5	5.5	> 30
2	> 30	23.5	14	> 30	> 30	25	18	20
3	> 30	26	30	> 30	> 30	6.5	14	> 30
4	> 30	27	12	16	17	8	14	17

Table III The effect of plasma and plasma fraction I on the Ivy bleeding time The bleeding time was measured before the infusion, and 15 min. 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Ivy bleeding time (min)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20-24 hrs	Before	15 min	4 hrs	20-24 hrs
1	> 30	30	> 30	> 30	> 30	17.5	24.5	> 30
2	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
3	> 30	> 30	> 30	> 30	> 30	17	> 30	> 30
4	> 30	26 27 30	> 20	> 30	> 30	13,20 > 30	18,30,>30	> 30
5	> 30	21,27 > 30	> 30	> 30	> 30	> 30	> 30	> 30

and infused into the patient. With this technique, the blood was rapidly chilled and exposed only to non wettable surfaces. The infusion started within one hour after collection of the blood

Preparation of Cohn s plasma fraction I Plasma, prepared as for infusion, was immediately cooled and precipitated with 8 ethanol, according to method VI of Cohn et al. (14) using siliconized equipment. After centrifugation, the precipitate was dissolved in 500 ml of a solution containing 0 9 NaCl and 0.6 sodium citrate, and immediately infused into the patient. The infusion always started within 4 hours of collection of the blood No side effects were observed

Hemostatic tests" were carried out by the techniques referred to in table I

Results

The hematocrit values decreased in proportion to the volumes infused The other results are presented in tables II—V Since the number of patients is small, we have not calculated mean values.

Duke bleeding time In all experiments there was a clear-cut decrease in the Duke bleeding time, but in only one patient did we obtain a bleeding time barely within the normal range (table II) The effect was marked during the first hours after infusion and could hardly be demonstrated after 20—24 hours. Fresh plasma and plasma fraction I had similar effects, but the response to

Table IV *The effect of plasma and plasma fraction I on the concentration of factor VIII Factor VIII was assayed before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I*

Patient no.	Concentration of factor VIII (%)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	19	56	53	32	7	30	53	44
2	5	55	32	41	2	20	17	14
3	7	54	62	50	50	71	73	39
4	45	86	77	64	50	61	86	63
5	7	21	34	23	12	19	38	16

Table V *The effect of plasma and plasma fraction I on the platelet count. The platelets were counted before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I*

Patient no.	Platelet counts (1,000/mm³)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	439	418	429	463	438	456	444	461
2	204	198	231	215	223	211	234	217
3	202	175	210	190	180	204	226	217
4	184	134	148	137	130	128	123	118
5	238	186	197	204	243	237	229	222

fraction I was somewhat greater There was no correlation between the shortening of the bleeding time and the amount of plasma infused or the concentration of factor VIII in the fraction I administered.

Ivy bleeding time The results are collected in table III The pattern of response was similar However the effect was very small indeed in three patients out of five 705—1 135 ml of fresh plasma had no effect t ll. In no case did the bleeding time become normal.

Factor VIII The results are given in table IV In all patients the concentration of factor VIII increased markedly and i some patients the increase was greater than expected on the basis of the amount infused. The activity disappeared more slowly than in transfused hemophiliacs. There was no parallel between the bleeding times and the factor VIII concentrations achieved and the effect on the bleeding time disappeared more quickly than the effect on factor VIII

Platelets. The platelet count decreased slightly probably due to hemodilution (table V) The decrease was too small to influence the bleeding time.

Table II The effect of plasma and plasma fraction I on the Duke bleeding time The bleeding time was measured before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Duke bleeding time (min)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	> 30	8	11	28	> 30	5	5.5	> 30
2	> 30	23.5	14	> 30	> 30	25	18	20
3	> 30	26	30	> 30	> 30	6.5	14	> 30
4	> 30	27	12	16	17	8	14	17

Table III The effect of plasma and plasma fraction I on the Ivy bleeding time The bleeding time was measured before the infusion, and 15 min 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Ivy bleeding time (min)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	> 30	30	> 30	> 30	> 30	17.5	24.5	> 30
2	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
3	> 30	> 30	> 30	> 30	> 30	17	> 30	> 30
4	> 30	26 27 30	> 20	> 30	> 30	13,20 > 30	18,30,>30	> 30
5	> 30	21,27 >30	> 30	> 30	> 30	> 30	> 30	> 30

and infused into the patient. With this technique, the blood was rapidly chilled and exposed only to non-wettable surfaces. The infusion started within one hour after collection of the blood.

Preparation of Cohn's plasma fraction I Plasma, prepared as for infusion, was immediately cooled and precipitated with 8 % ethanol, according to method VI of Cohn et al. (14) using siliconized equipment. After centrifugation, the precipitate was dissolved in 500 ml of a solution containing 0.9 NaCl and 0.6 sodium citrate, and immediately infused into the patient. The infusion always started within 4 hours of collection of the blood. No side effects were observed.

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duced either no or only a questionable effect.

These results raise three questions

a) What is the reason for the difference between the results obtained with the Ivy and Duke techniques? The Duke wound is smaller and deeper than the Ivy wound but this can hardly explain the difference. Nor can the difference be due to the pressure used in the Ivy method, since parallel tests with no pressure gave similar results. Thus, we do not know the reason for the difference, but our observations may explain some of the discrepancies in the literature.

b) Which method more truely reflects the hemostatic efficiency in a patient? This question cannot be answered at present, but the fact that patients have been safely operated upon after infusions of fraction I—O may suggest that the Duke bleeding time is a better practical guide than the Ivy bleeding time. We have also observed a family with prolonged Ivy bleeding time but no bleeding tendency (unpubl. observation) A comparison of the two techniques in patients with different bleeding disorders may throw further light on this problem.

c) Are our result compatible with the postulated "bleeding time factor"? The results with the Duke bleeding time do support the existence of such a factor The negative results with the Ivy bleeding time could be due to qualitative or quantitative differences in the effect of the "bleeding time factor" on the two methods. It appears unlikely that the hemostasis of the two types of wounds should be qualitatively different. It is also difficult to accept the explanation that too little plasma was given about one liter of fresh normal plasma ought to have had some effect. At present, therefore, both of these explanations appear unsatisfactory Although the positive results with the Duke bleeding time carry more weight than the negative with the Ivy method this discrepancy must be elucidated before the "bleeding time factor" can be finally accepted.

3 *The effect on factor VIII*

Nilsson et al. (33 35) and Cornu et al. (15) have observed that factor VIII increased more than expected and disappeared more slowly than in transfused hemophiliacs. Our results support these observations.

Summary

Five patients with von Willebrand's disease (prolonged bleeding time, reduced concentration of factor VIII and bleeding manifestations) were transfused with 705—1 135 ml of fresh citrated plasma collected without exposure to wettable surfaces, and also with 500 ml of Cohn's fraction I.

The Duke bleeding time became shorter in all experiments but in only one did it became normal. The Ivy bleeding time showed little or no response. The significance of these observations is discussed.

Factor VIII increased markedly and decreased more slowly than in transfused hemophiliacs.

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Discussion

1. *Therapeutic effect*

Many authors have reported a therapeutic effect of blood, plasma and plasma fractions on bleeding in patients with von Willebrand's disease (1, 5, 15, 17, 31, 33, 34, 37). Such observations are impressive at the bedside, but difficult to evaluate scientifically. There are also reports of little or no effect of transfusions (7, 12) and Pitney and Macfarlane (8) and Valberg and Brown (47) maintained that these patients often do not bleed profusely at operations, they bleed primarily from skin and mucous surfaces. It is possible that therapeutic failures could be explained on the basis of insufficient amounts transfused, nevertheless the clinical evidence appears to be suggestive only.

To illustrate the difficulties involved in the clinical observation of bleeding, reference can be made to the discussion on the effect of non-viable platelets in thrombocytopenia. Clinical experience strongly suggested a hemostatic effect of such platelets (29), but no effect was found in quantitative animal experiments (20).

2. *The effect on the bleeding time*

Nilsson et al. (33, 34, 35, 36, 37, 38) have shown that normal plasma and plasma fraction I—O normalize the prolonged Duke bleeding time in patients with von Willebrand's disease. A clear-cut dose-response relationship has not been reported. An ordinary blood transfusion had no effect. "half a dose" of fraction I—O (prepared from 600—700 ml fresh plasma) did not always correct the bleeding time, but one dose of fraction I—O or 400—800 ml fresh plasma normalized the bleeding time.

The effect lasted for 24—48 hours. It was not related to platelets, fibrinogen or factor VIII. Fraction I—O prepared from hemophilic plasma was effective (two experiments), but the same fraction prepared from the plasma of patients with von Willebrand's disease had no effect (one experiment). Based on this evidence they postulated that normal plasma contains a "bleeding time factor" which is lacking in von Willebrand's disease.

This effect of normal plasma on the bleeding time has been observed by many others (1, 2, 6, 15, 16, 30, 31, 42, 46, 47). There are also reports of no effect, but except for McMillan's (32) study they are less extensive than the positive reports (3, 7, 25, 28, 43, 44). Nearly all of these authors used the Duke method, two used the Ivy method (7, 25), and one used single stabs on the forearm (32). A few authors did not specify their method (3, 28, 42).

It could be argued that the effect might be unspecific, and this argument appears to be supported by the observation that fraction I stops bleeding and normalizes the bleeding time in thrombocytopenia (13, 30). However, Nilsson et al. (35) found no effect of fraction I—O in three patients with thrombocytopenia and in one patient with macroglobulinemia of Waldenström. Further, they found no effect of fraction I—O prepared from patient with von Willebrand's disease.

We found a definite effect on the Duke bleeding time of both fresh plasma and fraction I, but the effect was much smaller than that reported by the Swedish workers. Both plasma and fraction I were prepared as carefully and rapidly as possible. The effect on the Ivy bleeding time was considerably smaller, the infusion of about one liter of normal plasma pro-

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Hereditary Deficiency of NADPH$_2$-Methaemoglobin Reductase

By

J. Müller, K. Murawski, Z. Szymanowska, A. Koziorowski and L. Radwan

Two possible mechanisms responsible for congenital familial methaemoglobinaemia are known at present. One consists in a structural abnormality of the protein moiety of haemoglobin which consequently is not able to combine reversibly with oxygen and quickly becomes oxidized in presence of oxygen. A number of such abnormal haemoglobins (haemoglobins M) have been described. The other mechanism is associated with a deficiency of the methaemoglobin-reducing systems present in normal erythrocytes. Two enzyme systems (methaemoglobin reductases) are probably present in the human red cells, utilizing glucose and lactate as the hydrogen donor and linked to the reduced forms of nicotinamide-adenine dinucleotid phosphate (NADPH$_2$) or nicotinamide adenine dinucleotide (NADH) respectively (7). The first kind of defect (abnormal haemoglobin M) is transmitted as a codominant while the second (enzyme deficiency) as a recessive autosomal character. The present status of both kinds of the hereditary methaemoglobinaemias has been reviewed by Gerald (6) and recent advances concerning the newly discovered variants of haemoglobin M can be found in the paper of Betke (1).

Hereditary methaemoglobinaemia is a rare condition and the majority of reported cases are isolated sporadic ones the published pedigrees are not numerous, especially those which were investigated biochemically. The present paper reports on a family in which three members with deficient methaemoglobin reductase have been found in one generation and the nature of the enzymatic defect was studied.

Methods

The concentration of methaemoglobin in blood was measured by the method of Evelyn and Malloy (5). All samples were centrifuged before reading to avoid errors due to turbidity. Total haemoglobin concentration was determined using the cyanmethaemoglobin method

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Methods

The concentration of methaemoglobin in blood was measured by the method of Evelyn and Malloy (5). All samples were centrifuged before reading to avoid errors due to turbidity. Total haemoglobin concentration was determined using the cyanmethaemoglobin method

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Table I. The concentration of methaemoglobin in blood of the patient before and after intravenous methylene blue (MB)

Date	Methaemoglobin %
Jan.	
9	7.49
29	8.73
29	1.21
30	0
Feb.	
1	0
3	0
5	0
7	0.79
9	0.36
12	1.13
16	8.85
16	0

30 min. after MB.

(4). The oxygen content and saturation of blood was determined using the manometric method (11).

The ability of intraerythrocytic enzyme systems to reduce methaemoglobin was tested using a method modified from Pisciotta et al. (12) and Betke et al. (2). Freshly obtained red blood cells were washed three times with saline and incubated for 2.5 hours in 1.5 vol. of 5 mM sodium nitrite solution in saline. Then the cells were washed free from nitrite with saline and 0.155 M phosphate buffer pH 7.3 ratio 9:1 and their methaemoglobin content was estimated. One half of the washed cells was then resuspended in 1 vol. of 5 % glucose and the other in 1 vol. of saline buffer and 0.2 vol. of 2.5 % sodium lactate. Both samples were incubated at room temperature for 20—25 hours (that in lactate in an atmosphere of carbon monoxide) and the methaemoglobin concentration was determined several times during this period.

Starch gel electrophoresis was performed in a vertical apparatus (16) in borate buffer pH 8.5 in the case of oxyhaemoglobin (untreated haemolysate) or in phosphate buffer pH 7.0 after conversion of the pigment to methaemoglobin by the addition of an excess of potassium ferricyanide. The electrophoretic analysis of the polypeptide chains of haemoglobin was performed on starch gel in a glycine buffer pH 2.2 (18). The rate of spontaneous oxidation of haemoglobin was measured spectrophotometrically at 630 mμ in 0.133 M acetate buffer pH 4.5 at room temperature.

Foetal haemoglobin was determined by the one-minute alkali denaturation method of Singer et al. (15). For the analysis of reduced glutathione the method of Grunert and Phillips (8) was employed: the stability of reduced glutathione was tested after the incubation of blood for 2 hours at 37° C in presence of 5 mg acetylphenylhydrazine per ml (3) with 400 mg glucose added (17). Other methods were routine laboratory and clinical techniques.

Results

The patient was a 26-year-old male physical worker. The only complaints were easy fatigability, periodical headache, heartache, and palpitation. He had had these symptoms for five years. Mild cyanosis of the tongue, lips, earlobes, cheeks and nail beds was present without clubbing of the fingers. A faint systolic murmur was heard over the heart. The blood pressure was 150/90 mm Hg. X-ray examination of the lungs, ECG, cardiac catheterisation and spirometry were within normal limits. The haematocrit was 49—58 % and haemoglobin concentration 16—17 g % in repeated determinations.

Blood methaemoglobin estimation gave a value of 9 %. In repeated determinations of the oxygen saturation of blood values ranging from 83 to 91 were obtained. Methylene blue (1 mg per kg body weight) was given to the patient intravenously which caused a drop in methaemoglobin concentration to 1.2 % after 30 min, during the next 9 days no

methaemoglobin was found in the patient's blood. After 9 days methaemoglobin reappeared in the blood and rose slowly to the initial value of 9 % during the next 9 days (table I)

Starch gel electrophoresis of oxyhaemoglobin (pH 8.5) and methaemoglobin (pH 7.0) revealed no abnormal components. The electrophoretic behaviour of polypeptide chains of the patient's haemoglobin was the same as that of normal haemoglobin (18) The rate of spontaneous methaemoglobin formation at pH 4.5 (0.133 M acetate buffer) was the same for the haemoglobin from patient's blood and for normal pigment (k = 2.8 × 10- in both cases) The absorption spectrum of methaemoglobin and cyanmethaemoglobin (fig. 1) as well as the rate of conversion of methaemoglobin to cyanmethaemoglobin were the same for normal and patient's haemoglobins.

The ability of the patient's erythrocytes to reduce methaemoglobin was then tested as described under Methods. The red blood cells of the patient possessed the same reducing power toward methaemoglobin as normal erythrocytes when suspended in lactate (fig 2a) while no

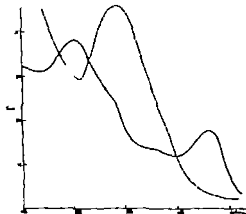
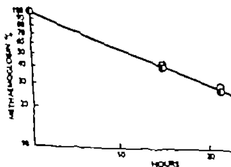


Fig. 1. Absorption spectrum of methaemoglobin (———) and cyanmethaemoglobin (— — —) of the patient. M/15 phosphate buffer pH 6.5.

methaemoglobin reduction in erythrocytes occurred in presence of glucose (fig 2b)

Blood samples from the patient's family were then tested and in two brothers of the propositus the same abnormality consisting in the inability to utilize glucose for methaemoglobin reduction was found. However no methaemoglobin has been found in their bloods. The remaining four brothers, the parents and an uncle who was available for study were apparently normal. The



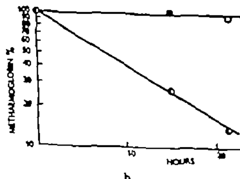


Fig 2 The rate of methaemoglobin reduction a) in lactate and b) in glucose in patient (○) and normal (●) erythrocytes.

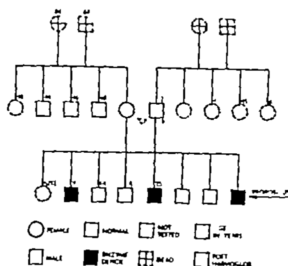


Fig 3 The pedigree of the patient's family

pedigree of the family under study is shown in fig 3

Foetal haemoglobin has been determined in some members of the family The results shown in the pedigree (fig 3) indicate that increased amounts of Hb F were found only in enzyme-deficient subjects. The glutathione stability test performed on erythrocytes from the patient was normal (54 9 before and 44 6 mg % after incubation with acetyl phenylhydrazine)

Discussion

The inborn error in the methaemoglobin reducing system found in the propositus and his two brothers seems to be very similar to that described by Townes and Lovell (19) and Townes and Morrison (20) However a dominant inheritance has been suggested by the above authors on the basis of reported cyanosis in their patient's father and grandfather whereas the family tree of our patient (fig 3) indicates a recessive transmission of the enzymatic defect. Further Townes and Morrison (20) reported an inadequate synthesis of glutathione in their patient's red cells and considered that this deficiency results in insufficient reduction of NAD thus impairing the $NADH_2$-dependent reductase. This likewise does not hold for our case where the concentration of reduced glutathione in erythrocytes was normal

The most probable explanation of the biochemical findings in the case described above is to assume that two methaemoglobin reducing systems are present in human red cells, one active toward $NADPH_2$ and the other toward $NADH_2$, utilizing glucose (in the hexose monophosphate shunt) and lactate respectively as a primary source of hydrogen (7) The NADPH reductase was isolated from normal human erythrocytes by Huennekens et al (9) $NADH_2$-reductase was shown to be absent in erythrocytes from a few cases with hereditary methaemoglobinaemia (13) It seems that either one of the two reducing systems may be hereditarily deficient with consequent congenital methaemoglobinaemia, and that the inability to utilize glucose for methaemoglobin reduction and a normal reduction in presence of lactate, as found in our case, means the absence of one of the reductases only namely that active toward $NADPH_2$. Only slight methaemoglobinaemia which was very sensitive toward the action of methylene blue was found in the propositus and no methaemoglobin was present (in single determinations, however) in the blood of his affected relatives. The hexose monophosphate shunt in which NADP is reduced accounts, however for only a small proportion of the glucose utilized by the red blood cell (10) and therefore lack of NADPH reductase might cause a smaller degree of methaemoglobinaemia than deficient NADH reductase. It should be also noted that the intensity of

methaemoglobinaemia varies with the diet and is markedly influenced by the ascorbic acid intake (14)

The repeated observations of a lowered oxygen saturation of the arterial blood in patients having normal cardiopulmonary function and no signs of a right-to-left shunt could be due to a shift to the right of the oxyhaemoglobin dissociation curve. This problem will be investigated in the near future.

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A family with deficient ability of erythrocytes to utilize glucose for met haemoglobin reduction has been de scribed. The methaemoglobin reduction in presence of lactate was normal and therefore the lack of one of the met haemoglobin reductases, that active toward the reduced form of nicotinamide adenine dinucleotide phosphate formed in the hexose monophosphate shunt, is suggested to be inherited. The family study indicates a recessive transmission of this defect. In two of the three enzyme-deficient subjects a slight elevation of foetal haemoglobin was found.

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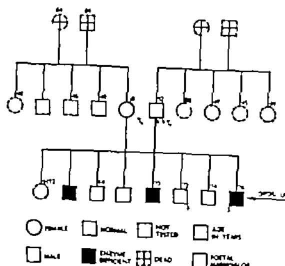


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From King Gustaf V Research Institute, Stockholm, Sweden

Are the Macroglobulins Giving Rise to a Positive Sheep Cell Test in Different Diseases Identical?

Preliminary Report

By

NANNA SVARTZ and STIG HEDMAN

As is nowadays well known, there are several diseases other than rheumatoid arthritis, which show a positive sheep cell test, although with much lower frequency. The question arises whether the macroglobulins of different diseases giving rise to a positive sheep cell test are identical, for instance the macroglobulin in rheumatoid arthritis (R. A.) and that in lupus erythematosus disseminatus (L. E. D.).

Since 1953 Svartz has on several occasions expressed the opinion that differences in the hemagglutinating factor can be demonstrated between R. A. and for instance L. E. D. (1). Some dissimilarities are brought to light even by such a simple method as precipitation in the cold (2, 3).

Theoretically *as well as clinically* it would be very important to know whether or not the different hemagglutinating macroglobulins are specific for one disease. If they are not identical, they might perhaps in future be used for diagnostic purposes. In this connection it is interesting to note that most sera from normal guinea pigs have the capacity of producing a positive sheep cell test. The macroglobulin in guinea pig serum does not seem to be identical with the rheumatoid factor.

We shall give a brief account of some studies of macroglobulins which have a. o. been reported in a "Morris Fishbein lecture" held in Chicago Dec. 3, 1962. We have tried by means of ultracentrifugation to discover whether differences can be shown to exist between the hemagglutinating substances in different conditions. Table I shows the results.

The cases of R. A. have a sedimentation coefficient of 18.6—18.9 S. They show a high hemagglutination titer in the sheep cell test and in the redissolved cold precipitate from the same serum. The percentage of macroglobulin in the total serum protein is also tabulated. The *amount of serum protein was usually about 7 %*.

The sera of patients suffering from L. E. D. and polyarteritis nodosa showed a macroglobulin having a sedimentation coefficient of 18.0—18.25 S. The sheep cell titers are 1:1,024 and 1:4,096, but the cold precipitate test showed titers of only 1:16 to 1:64. Thus, both the sedimentation coefficient and the cold pre-

Submitted for publication January 7, 1963.

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1	R. A.	18.8	4 1	1 : 32,768	1 : 16,384
2	R. A.	19.3	5.6	1 : 4,096	1 : 4,096
3	R. A.	18.7	10.9	1 : 4,096	1 2,048
4	R. A.	18.65	6.1	1 : 8,192	1 : 4,096
5	R. A.	18.6	5.5	1 4,096	1 2,048
6	R. A.	18.7	5.4	1 : 16,384	1 : 8,192
7	R. A.	18.6	5.3	1 : 16,384	1 : 8,192
8	L. E. D	18.1	4.0	1 1 024	1 : 16
9	L. E. D	18.25	7 1	1 : 1,024	1 16
10	L. E. D	18.0	6.6	1 : 4 096	1 : 32
11	Polyarterit. nodosa	18.0	6.4	1 1,024	1 : 64
12	Collagen dis. (?) Thrombo-phlebitis	17 75	7 4	1 : 512	0
Guinea-pig	Healthy	20.2	8.9	1 : 128	0
Guinea-pig	Healthy	20 1	7.5	1 : 16	0
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cipitate test differed from the results obtained in R. A. In this connection we must add that there are some sera from patients diagnosed as L. E. D that behave in the same way as R. A. sera but it is not quite clear whether the L. E. D in those cases was a true one.

On the other hand the guinea pig sera showed a higher S-value than the R. A. and L. E. D.-sera but the sheep cell test showed only low titers. The cold precipitate showed a negative hemagglutination reaction.

Myeloma sera sometimes contain a macroglobulin with a high S-value, but nevertheless often do not show a positive hemagglutination test or show only a very weak one. The same holds for the macroglobulin in the blood of healthy subjects.

Summary

In comparing the sedimentation coefficients, the hemagglutinating capacity and the precipitation in cold it is possible to some extent to draw conclusions concerning the type of disease. In our opinion ultracentrifugation if the centrifuge and methods are well standardized gives the most valuable information. However further checks are to be made.

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From the Department of Medicine, University of Lund, Malmö General Hospital
(Head Jan Waldenström, M D) Malmö, Sweden

Supplemental Triiodothyronine in the Treatment of Constipation of Hypothyroidism Resistant to Desiccated Thyroid

By

Bengt Skanse

It has been claimed that the effect of triiodothyronine (T_3) does not differ in type from that of desiccated thyroid or sodium l thyroxine (T) except that the response to T is prompter (2, 9 10) Frawley et al. (1) described a case of hypothyroidism resistant to thyroid extract but responsive to T_3. Newman and Escamilla (8) found that T or the combination of T and desiccated thyroid or T sometimes had a better effect on hypothyroidism than desiccated thyroid or T alone. Jefferies (5) has observed patients with hypothyroidism who were resistant to desiccated thyroid but not to T or T_3.

The purpose of the present investigation was to study T and desiccated thyroid for any difference in effect on obstinate constipation of hypothyroidism.

Material and methods

Seven female patients with hypothyroidism, 6 with idiopathic and 1 with postoperative hypothyroidism (case 1), form the basis of the report. All of the patients had had hypothyroidism for more than 5 years and all had received treatment with desiccated thyroid for at least 3 years. Their symptoms and signs of hypothyroidism had responded to treatment with the exception of the constipation (all 7 patients) and the dryness of the skin (cases 2 and 4) The basal metabolic rates ranged between —13 and +5 during treatment. The doses of desiccated thyroid required to control the hypothyroid symptoms, except for those mentioned above varied between 180 and 360 mg per day In an attempt to correct the obstinate constipation, the dose of thyroid extract was increased by 240—300 mg, i. to 480—600 mg per day for 4—12 weeks. This resulted in symptoms and signs of overdosage, particularly nervousness, restlessness, palpitation, and increased sweating and headache, but it had no beneficial effect on the constipation. The doses of thyroid were therefore reduced to the original level, or to 60 mg abov that level, except in case 2 where the dose was kept t 180 mg above the original level before the trial with T was started. The dose of thyroid extract was then kept constant in each patient during the rest of the study (table I)

The patients were informed that the purpose of the study was to compare two thyroid preparations. T and placebo tablets were dispensed by the usual double-blind

Submitted for publication August 20, 1962.

Erco Pharmaceutical Company generously supplied triiodothyronine.

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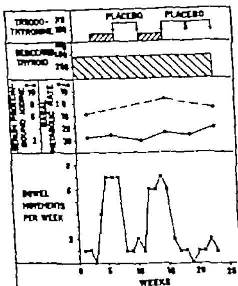


Fig 1 The effect of supplemental triiodothyronine on the frequency of bowel movements in case 1 (25 years of age)

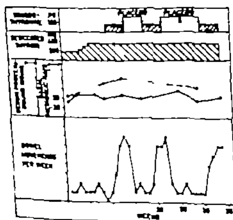


Fig. 2. The effect of supplemental triiodothyronine on the frequency of bowel movements in case 2 (42 years of age)

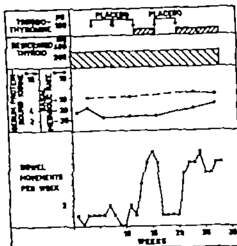


Fig 3. The effect of supplemental triiodothyronine on the frequency of bowel movements in case 5 (38 years of age)

increase of the BMR (fig) or the pulse rate was noted. The dryness of the skin of case 4 but not of case 2 improved during treatment with T_3.

The 2 patients (Nos. 6 and 7) in whom no effect on constipation was observed during the double-blind trial were later given a larger dose of T (no. 6 120 µg/day and no. 7 160 µg/day) for 4 weeks. This resulted in characteristic symptoms of thyroid overdosage but had no effect on the constipation.

Follow-up of the patients for 6 months to 4 years showed that combined T and thyroid extract had kept them free from constipation.

Discussion

Constipation is common in hypothyroidism, and according to Means (7) it occurs in 61 % (Lerman's series of 77 cases). The constipation usually responds to treatment with thyroid extract to the same extent as other symptoms and signs of hypothyroidism. Sometimes, however this symptom may be rather resistant to therapy

It cannot be concluded from the present trial whether the constipation would have responded to T alone. Investigation of this particular problem

Table I. Effect on constipation of supplemental treatment with triiodothyronine

Patient No.	Dose of desiccated thyroid mg/day	Dose of triiodothyronine μg/day	Protein-bound iodine μg/100 ml	Bowel movements per week							
				Before experiment	During triiodothyronine			During placebo			
					1st period	2nd period	3rd period	1st period	2nd period	3rd period	4th period
1	360	60	6.3— 8.6	0.7	6.3 (1)	6.3 (3)	—	1.3 (2)	1.0 (4)	1.3 (5)	—
2	360	80	4 4— 8.3	1 0	5.5 (1)	5.3 (3)	5.3 (6)	1.3 (2)	1.5 (4)	1.0 (5)	—
3	240	60	6.4—11.2	1.0	6.0 (2)	7 0 (3)	5.5 (4)	0.8 (1)	1.0 (5)	1.3 (6)	—
4	300	60	6.3— 6.8	0.8	4.5 (1)	5.5 (3)	5.3 (4)	1.3 (2)	1.0 (5)	—	—
5	240	60	6.3— 7.2	0.8	5.8 (3)	6.0 (5)	5.5 (6)	1.3 (1)	0.8 (2)	1.0 (4)	—
6	300	60	3.9— 7.8	1.0	1 0 (4)	1.5 (5)	—	1.0 (1)	0.8 (2)	0.8 (3)	1.0 (6)
7	240	80	5.2— 6.4	0.8	1.0 (2)	1.0 (4)	0.8 (5)	1.0 (1)	0.8 (3)	0.8 (6)	—

Postoperative hypothyroidism.
Figures in parentheses give the number of the experimental period in the individual patient.

method for periods of 4 weeks at a time. The dose of T administered was 60 or 80 μg per day, usually 60 (table I). The dose of T in a given case was smaller than the equivalent amount of the supplemental desiccated thyroid that had failed to alleviate the constipation. A dose of 30 μg of l-triiodothyronine was taken as being equivalent to 65 mg of desiccated thyroid (6). The entire dose of desiccated thyroid, T or placebo was given in the morning. The trial was continued for five or six 4-week periods (table I). All patients were treated on an out-patient basis.

The patients were instructed not to change their way of living or dietary habits during the experimental period. They were also requested not to use laxatives. All except 3 reported that they had followed their instructions. These 3, of whom 2 had responded favourably, were therefore not included in the present investigation. The patients were also instructed to make a note of the number of bowel movements for each week, and of any other particular effects. The basal metabolic rate and the serum protein-bound iodine were determined at various intervals, usually once every 4-week period of treatment. It was noticed that the body weight did not vary much during the experiment.

Results

The results are shown in table I. Detailed data for 3 patients are presented in figs. 1—3. A significant increase occurred in the frequency of bowel movements in 5 patients, but not in the remaining 2. In these 5 patients the number of bowel movements increased from an average of 0.9 per week to 5.8 per week. T_3 did not produce its full effect on constipation until a few days after treatment had been started (figs. 1—3).

The administration of T_3 had no significant effect on the BMR. The serum protein-bound iodine values ranged from 3.9 to 11.2 μg/100 ml (all values obtained during treatment). As a rule, the pulse rate did not increase significantly except in cases 6 and 7, in whom T had no effect on the constipation. These two patients also developed nervousness, increased sweating and headache. In addition case 2 reported restlessness during the administration of T_3, but no

thyroid argues against the hypothesis of only a quantitative difference and may suggest some difference in type of action. One might also imagine T to have a permissive action in the sense suggested by Ingle (4) for adrenocortical hormones.

It may appear somewhat surprising that, as in some patients in Newman and Escamilla's series, supplemental T in doses of 60—80 µg per day produced no further rise in the BMR. The reason for this lack of effect is not clear

In the light of the considerations discussed above it appears that T might be worth trying in constipation of hypothyroidism resistant to thyroid extract.

Summary

In 7 hypothyroid patients with obstinate constipation resistant to treatment with desiccated thyroid even in excessive dosage supplemental T in doses of 60—80 µg per day controlled the constipation in 5 of the 7 patients. The observations would support the thesis that the sensitivity to thyroid hormones may differ from one organ to another The beneficial effect of combined T and desiccated thyroid but not of desiccated thyroid alone suggests the possibility of differences in the mechanism of action of T and desiccated thyroid.

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would not have been possible without undue inconvenience to the patients because of rapid development of hypothyroid symptoms during administration of only placebos

The lack of any response of the constipation to combined desiccated thyroid and T_3 in 2 of the patients may have been due to the condition being of some origin other than hypothyroidism. However no such etiological factor could be demonstrated It should also be mentioned that I have never found treatment of non hypothyroid constipation with T_3 to be successful.

It is still debatable whether the metabolic disturbance in hypothyroidism differs in severity from one organ to another However even if such a difference does exist, it can hardly be held reponsible for the refractoriness of constipation in the present patients since increasing the doses of thyroid extract up to an amount that produced symptoms and signs of overdosage in all patients studied produced no alleviation of the constipation. Nor could the lack of response to thyroid be ascribed to impaired absorption of the drug since the serum protein-bound iodine values during treatment were either normal or high (table I) In addition the patients developed symptoms of thyroid overdosage, which they would not have done if the absorption had been seriously impaired

The varying response of constipation to treatment with thyroid hormones might be explained by differences in the sensitivity of various organs to the hormone. Although no definite evidence is available for such an assumption there is support from the fact that thyroid extract had a beneficial effect on all of the symptoms except constipation and dryness of the skin (2 patients) The observations made by Newman and Escamilla also suggest that different symptoms of hypothyroidism may vary in their response to thyroid extract, to T_3 or to a combination of both. However since the doses of T_3 used in their investigation were not always equivalent to the doses of thyroid comparison with the present data is difficult. It is thus not possible to gather from their paper whether constipation responded better to T_3 alone or to a combination of T_3 and thyroid extract than to desiccated thyroid alone In the present series differences in end-organ sensitivity might explain why the constipation of hypothyroidism responded in some patients but not in others. No clinical characteristics permitting prediction of the effect of addition of T_3 were observed The hypothesis of differences in sensitivity of various tissues to thyroid hormone is also supported by the observation made in patient no 2 in whom supplemental T_3 controlled the constipation but had no effect on the dryness of the skin and hair Furthermore the findings of Hutchison, Arneil and McGirr (3) in a case of non-goitrous sporadic cretinism suggest that some patients may be refractory to thyroid extract but respond well to T_3. They postulated that such a type of hypothyroidism might be due to an inborn deficiency of an enzyme concerned with the peripheral deiodination of T to T

The favourable effect of supplemental T_3 on the constipation in some hypothyroid patients may also be tentatively explained by differences in mode of action of T and desiccated thyroid. The fact that some patients who had not responded to an even larger equivalent dose of desiccated thyroid alone but responded to combined T and desiccated

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Exercise Electrocardiograms in a 5-year Follow-up Study

By

Irma Åstrand

Typical "ischemic" electrocardiographic changes are important in diagnosing coronary heart disease. This was stressed by among others, Biörck (4) Yu and Soffer (22) Simonson and Keys (18) Bengtsson (3) Frick et al. (7) Abarquez et al. (1) Dimond (6) and Sandberg (16) It has been emphasized that it is important to record the ECG not only after but also *during* exercise (2, 3 25) Multiple recordings during work at *increasing work loads* are also more informative than a single tracing obtained at a submaximal work load (25) The significance of minor electrocardiographic changes appearing at rest or with exercise has, however not been established.

In an attempt to elucidate the validity of ECG changes appearing during and after work (exercise ECG) a group of 73 males was examined twice with a 5-year interval. From a clinical and clinico-physiological point of view the main points studied were the following

1 The frequency of exercise ECG changes found on the re-examination in subjects with a normal ECG originally

2. Accentuated changes in the exercise ECG:s in subjects with minor ECG changes five years earlier

3. Symptoms in subjects with a definitely pathological exercise ECG five years previously

4. The relation between symptoms and ECG changes.

Material

During 1957 81 out of group of 100 male truck drivers 50 to 64 years old were examined (examination I) (23, 24) They were employees at three Stockholm breweries and their work was comparatively heavy During the 5-year follow-up period, 4 of the 81 died. For different reasons 4 subjects did not want to take part in the re-examination of 1962 (examination II) thus leaving 73 subjects in the series (55 to 70 years old), see table I Of the 73 subjects, one was receiving digitalis therapy (decompensated, S-T T depressions at rest) while several others were on antihypertensive drug treatment. Five had slight anemia (< 12.7 g % hemoglobin at examination II) without S-T T changes t rest or work. Forty-six subjects had the same daily work at examination I and II. At the time

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Book review

Drugs of choice 1962—1963 Edited by Walter Modell. 941 pp Price $14.50 The C. V Mosby Co St. Louis, Miss. 1962

A new edition of this work every year ensures that effect is given to its aim of presenting the medical profession with "authoritative and unbiased information on the choice of a particular drug for a particular clinical situation"

The planning and the structure of the book are strictly in accordance with earlier editions. In 41 chapters 48 experts on different fields give short but comprehensive descriptions of the pharmacotherapy of most common diseases. All chapters have been revised and some have been completely re written A new chapter on diabetes has been added while the chapter on the use of tranquilisers in the preceding edition has been considered too highly specialised and is left out.

Like the earlier issues, this one appears well edited so that a certain consistency is kept in spite of the many different authors. The book ends with a list of about 3 500 drugs with generic or trade names, a true choice of drugs. Compared with the former edition, 150 new drugs have appeared including chloraminodiazepoxide and amitriptylene. A few drugs from earlier editions have disappeared mostly because of change of name. Most of the drugs are American only about 20 preparations are named which are not commercially available in the U S A. most of them being Canadian. Few if any European drugs not obtainable in America are included in the list.

The book is a worthy successor of the earlier editions.

Erik Jacobsen

Copenhagen

ECG The ECG code used is mainly in agreement with the Minnesota code for resting ECG (5). Certain additions to the code were made regarding the S-T junction and segment, the T-wave items and the arrhythmias, see table II. This was done to allow coding of and differentiation between minor changes, which is of particular value when analysing the results of re-examinations. The somewhat modified code was applied to all ECGs including exercise and post-exercise records. Q and QS patterns, QRS axis deviation and high amplitude R waves, however were coded only in the resting ECG records (V-leads)

Whether the use of CH and CR-leads during and after exercise instead of modified V-leads resulted in any difference in classification is an open question. That CR and CH-leads agree very closely and thus are *interchangeable* has been shown by Holmgren and Strandell (8)

It is important during exercise to measure depressions of S-T junction and segment from preceding PR interval at onset of QRS because of elevation of the isoelectric line

That an S-T depression of the strictly junctional type frequently occurs in clinically healthy subjects and that the degree of the S-T depression increases gradually with increasing work loads and heart rate has been shown by Sjöstrand (20) and Sandberg (16) Such changes disappear quickly after work when the heart rate reverts to the resting level (15) Horizontal or downward sloping S-T segments appeared in this study both during and after work and were usually most pronounced 3 min. after work They were closely correlated to symptoms, which strongly suggests that the *shape of the S-T segment* always should be considered. This is mainly in agreement with the findings of Bengtsson (3) and Lepeschkin and Surawicz (11) In the classification of the S-T changes in the exercise ECG as whole the junctional changes were finally grouped as 1 or 2 depending upon the degree of the depressions and whether they persisted or not 3 min. after work The horizontal or downward sloping S-T segment were grouped in the same w y according to severity as 3, 4 or 5 (table II)

A decreased T wa amplitude has also been found in clinically health subjects on exercise (10, 16, 17 19) In the present study the T wave changes were alw ys accom-

Table II. Modifications of the Minnesota ECG code (5), and corresponding severity grouping

Code	Punch	Severity group	*S-T junction and segment (measured from preceding PR interval at onset of QRS) Leads I or II VF V V or CR_1-CR_6*
IV	1	5	S-T J depr 0.6 mm or more *and* S-T segm. downward sloping or S-T J depr 1 mm or more *and* S-T segm. horizontal
	2	4	S-T J depr 0.6–0.9 mm *and* S-T segm. *horizontal*
	3	4	No S-T J depression as much as 0.5 mm *but* S-T segment sloping down and reaching *0.5* mm or more below PR baseline
	4	3	N S-T J depression as much as 0.5 mm *but* S-T segment horizontal or downward sloping but not reaching 0.5 mm below the PR baseline
	5	2	S-T J depression 1 mm or more
	6	1	S-T J depression 0.6–0.9 mm
	7	—	S-T segment elevation of 2.0 mm or more
			T wa items
V	1–3	—	As in the original code
	4	—	Low amplitude T-waves
			Arrhythmias
IX	1	5 (b)	Bigeminal, trigeminal or quadrigeminal entricular ectopic beats (VES)
		5 ( )	Frequent (4 or more in 40 complexes) VES and supraventricular ectopic beats (SVES)
	3	4	Frequent VES
	4	3	Frequent SVES
	5	2)	Occasional ES less than 4 in 40 compl. VES and SVES
	6	2 b	Occasional VES
	7	2)	Occasional SVES or ectopic trial rhythm

Table I Frequency of overweight and high blood pressure by age at examination II

Age (years)	No.	No. of persons with			
		Over weight	Normal- or under weight	High B. P	Normal B. P
55—59	44	11	33	19	25
60—64	23	9	14	10	13
65—70	6	1	5	3	3
Total	73	21	52	32	41

of examination II 3 had retired because of disability 3 because of age and 21 had changed to lighter work.

Methods

The methods described here are only slightly modified from those used in examination I

A *medical history* was taken and a *physical examination* was made with the usual technique with particular emphasis on the chest organs.

After 10—15 min in a recumbent position the *blood pressure* was measured on the left arm using a mercury manometer If the systolic pressure was higher than 160 mm Hg and/or the diastolic higher than 100 mm Hg it was re measured at least twice and the lowest value obtained was used in the calculations.

The *body weight* and *body height* were measured

The *physical work* was performed on a bicycle ergometer at stepwise increased work loads with a work period of 6 min. at each level, usually two 600 and 900 kpm/min. respectively The pedal rat was about 60/min. In cases with symptoms likely to signify a reduced physical work capacity the work started at a lower load The work was interrupted at a heart rate of about 150/min. (higher than 144 in 51 cases) unless symptoms or the development of definitely pathological ECG changes moti ated a termination at a lower heart rate.

The *ECG* was recorded *at rest* before about 1 3, and 10 min respectively *after cessation of the work* using leads I II III CR_1, CR_2, CR_4, CR_5 and CR at rest before also using leads aVR, aVL, aVF V_1 V_2 V V_5 and V *During* and immediately after that the work was stopped, the ECG was recorded with leads CH_1, CH_2, CH and CH_7 (H = head) During exercise ECG was usually recorded at the 2nd, 4th, 5th and 6th min. on each load

The *heart rate* was determined from the ECG recorded during the last three consecutive minutes. If there was a further increase in rate, then the maximum was used.

The *blood lactic acid concentration* was determined according to a modification (1949) of Barker and Summerson s method. Blood samples were drawn from a fingertip immediately after and about 3 min. after the work was stopped. The highest value was used.

Evaluation of the results

Blood pressure Subjects with a blood pressure of > 160 mm of Hg systolic and/or > 100 mm Hg diastolic pressure were classed as having high blood pressure.

Body weight A person was considered overweight if his weight in kg exceeded his body height in cm over 100 cm by more than 10

Symptoms The symptoms which the subjects described when their medical histories were taken were classified into 6 groups (A—F). A = palpitations or awareness of the heart at rest. B = chest symptoms other than A and C (angina pectoris) at rest and/or during work. D = effort dyspnoea which had increased during the last year before the examination. E = symptoms and/or signs of cardiac decompensation. F = was receiving or had received benefit payments for some time during the last two years because of symptoms of high blood pressure.

Angina pectoris was diagnosed according to the main criteria of Rose (14) (the pain or discomfort must nclude sternum *or* left anterior chest *and* left arm. Pain must be provoked by either hurrying or walking uphill and it must be of such intensity that it causes the person to stop or to slacken his pace) When the pain or the discomfort d d not meet these criteria the symptoms were referred to group B.

ECG. The ECG code used is mainly in agreement with the Minnesota code for resting ECG (5). Certain additions to the code were made regarding the S-T junction and segment, the T-wave items and the arrhythmias, see table II. This was done to allow coding of and differentiation between minor changes, which is of particular value when analysing the results of re-examinations. The somewhat modified code was applied to all ECGs including exercise and post-exercise records. Q and QS patterns, QRS axis deviation and high amplitude R waves, however, were coded only in the resting ECG records (V-leads).

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	5	2	Occasional ES (less than 4 in 40 compl.) VES and SVES
	6	2 b	Occasional VES
	7	2)	Occasional SVES or ectopic atrial rhythm

Table III Frequency of various symptoms A—F at examination I and II The underlined numbers refer to subjects with high blood pressure

Examination I

Examination II	No symptoms	A	B	C	D	E	Total
No symptoms	31 12	— 2	2 1	— —	— —	— —	33 15
A	1 —	— —	— —	— —	— —	— —	1 0
B	— 1	2 —	— —	— —	— —	— —	2 1
C	2 3	— —	1 —	1 1	— —	— —	4 6
D	1 1	— 1	— —	— —	— 1	— —	1 3
E	— 2	— —	— —	— —	— —	— 1	0 3
F	— 3	— 1	— —	— —	— —	— —	0 4
Total	35 22	2 6	3 1	1 1	0 1	0 1	41 32

panied by changes in the S-T segment. Lepeschkin has stressed (10) that the S-T changes reveal more information about the coronary blood flow than the T changes do To simplify the presentation of the results, the T wave changes have been included under code IV *groups 1—5* together with the S-T changes.

The maximum degree of arrhythmia at rest, at any level of work or at any occasion post exercise was coded. The code was simplified and the arrhythmias grouped as 2 3 4 or 5 (table II)

Results

Four subjects died during the follow up period one of congestive heart failure which was already manifest at examination I two of bronchial carcinoma, one of myocardial infarction. This last subject had high blood pressure and was over weight, and he had symptoms of type D but neither S-T T changes nor ES in the ECG from examination I

It is well known that high blood pressure and obesity are predisposing factors in coronary heart disease. This material has therefore been analysed with regard to high blood pressure and overweight.

a. Blood pressure and body weight

At examination I 46 subjects had normal blood pressure and 27 had high blood pressure at examination II the corresponding numbers were 41 and 32 respectively Five subjects had borderline blood pressures at examination I and high blood pressures at examination II. In the tables where the two groups of subjects with normal and high blood pressures respectively are compared at the two examinations these 5 subjects are classified as having high blood pressures at both occasions.

Symptoms were significantly more frequent in the group of subjects with high blood pressure ($0.01 > P > 0.001$ see table III)

The frequency of high blood pressure is higher in the group with overweight than in the group of normal- or under weight subjects. The difference is statistically probably significant ($0.05 > P > 0.01$)

b ECG changes — symptoms

Arrhythmias (Code IX, table II) At rest at examination I 3 persons had VES (ventricular ectopic beats) 4 had SVES (supraventricular ectopic beats) and 1 had an ectopic atrial rhythm. At examination II 3 had VES 7 had SVES and 1 had both VES and SVES. Of

Table IV Frequency of ES or ectopic atrial rhythm with exercise at examination I and II

Examination I

Examination II: Code-group	1	2	2b	2c	3	4	Total
1	—	3	8	—	2	1	14
2	3	1	2	2	—	—	10
2b	3	—	2	—	—	1	6
2	—	—	1	—	—	1	2
3	2	—	2	—	—	1	5
4	2	1	1	—	—	1	5
5	3	—	—	—	—	1	4
5b	—	—	2	—	—	—	2
Total	13	5	18	2	2	6	48

1 = no arrhythmia.
2 = occasional (less than 4 in 40 complexes) SVES or ectopic atrial rhythm.
2b = occasional VES.
2 = occasional ES, VES and SVES
3 = frequent (4 or more in 40 complexes) SVES.
4 = frequent VES.
5 = frequent VES and SVES.
5b = bigeminal, trigeminal or quadrigeminal VES.

Table V Relation between ES and S-T T changes of different severity groups (see table II) in the exercise ECG

Examination I (1957)

ES-group

S-T T group	1	2	3	4	Total
1	26	16	—	3	45
2	2	2	1	—	5
3	3	5	1	1	10
4	8	1	—	2	11
5	1	1	—	—	2
Total	40	25	2	6	73

Examination II (1962)

ES-group

S-T T group	1	2	3	4	5	Total
1	19	8	2	3	1	34
2	1	2	1	—	3	7
3	3	1	—	—	1	5
4	6	—	1	—	1	8
5	10	6	1	2	—	19
Total	39	18	5	5	6	73

these only 2 and 4 respectively had these arrhythmias only at rest

In the exercise ECG records there were at examination I 33 and at examination II 34 (bout 45) subjects who had ES or an ectopic atrial rhythm (for details see table IV) Of these 27 and 26 respectively had the arrhythmia only on exercise In these respects there was no difference between the two blood pressure groups. Altogether 48 (66) subjects had ES at one or both examinations.

Both the type and the frequency of ES may vary individually as is evident when examination I is compared with examination II (see table IV) From table V it is evident that there is no correlation between the degree of S-T T depression and the frequency of ES in the exercise ECG records.

Two subjects exhibited accelerated conduction (WPW) during exercise at examination II None of them had any symptoms.

S-T J and segment and T wave item changes

ECG at rest. Table VI shows how the individual S-T T items changed between examination I and II Among the subjects with a *normal blood pressure* at examination I there was one subject with group 2 and one with group 5 S-T T

Table III Frequency of various symptoms A—F at examination I and II The underlined numbers refer to subjects with high blood pressure

Examination I

Examination II

	No symptoms	A	B	C	D	E	Total
No symptoms	31 12	— 2	2 1	— —	— —	— —	33 15
A	1 —	— —	— —	— —	— —	— —	1 0
B	— 1	2 —	— —	— —	— —	— —	2 1
C	2 3	— 2	1 —	1 1	— —	— —	4 6
D	1 1	— 1	— —	— —	— 1	— —	1 3
E	— 2	— —	— —	— —	— —	— 1	0 3
F	— 3	— 1	— —	— —	— —	— —	0 4
Total	35 22	2 6	3 1	1 1	0 1	0 1	41 32

panied by changes in the S-T segment. Lepeschkin has stressed (10) that the S-T changes reveal more information about the coronary blood flow than the T changes do. To simplify the presentation of the results, the T wave changes have been included under code IV *groups 1—5* together with the S-T changes.

The maximum degree of arrhythmia at rest, at any level of work or at any occasion post exercise was coded The code was simplified and the arrhythmias grouped as 2 3 4 or 5 (table II)

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It is well known that high blood pressure and obesity are predisposing factors in coronary heart disease. This material has therefore been analysed with regard to high blood pressure and overweight.

a Blood pressure and body weight

At examination I 46 subjects had normal blood pressure and 27 had high blood pressure at examination II the corresponding numbers were 41 and 32 respectively Five subjects had border line blood pressures at examination I and high blood pressures at examination II. In the tables where the two groups of subjects with normal and high blood pressures respectively are compared at the two examinations, these 5 subjects are classified as having high blood pressures at both occasions.

Symptoms were significantly more frequent in the group of subjects with high blood pressure (0 01 > P > 0.001 see table III)

The frequency of high blood pressure is higher in the group with overweight than in the group of normal- or under weight subjects. The difference is statistically probably significant (0 05 > P > 0 01)

b ECG changes — symptoms

Arrhythmias (Code IX, table II) At rest at examination I 3 persons had VES (ventricular ectopic beats) 4 had SVES (supraventricular ectopic beats) and 1 had an ectopic atrial rhythm. At examination II 3 had VES, 7 had SVES and 1 had both VES and SVES. Of

Table IV Frequency of ES or ectopic atrial rhythm with exercise at examination I and II

Examination I (columns); Examination II (rows)

Code-group	1	2	2b	2	3	4	Total
1	–	3	8	–	2	1	14
2	5	1	2	2	–	–	10
2b	3	–	2	–	–	1	6
2	–	–	1	–	–	1	2
3	2	–	2	–	–	1	5
4	2	1	1	–	–	1	5
5	3	–	–	–	–	1	4
5b	–	–	2	–	–	–	2
Total	15	5	18	2	2	6	48

1 = no arrhythmia.
2 = occasional (less than 4 in 40 complexes) SVES or ectopic atrial rhythm.
2b = occasional VES.
2 = occasional ES, VES and SVES
3 = frequent (4 or more in 40 complexes) SVES.
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Examination I (1957)

ES-group (columns); S-T T group (rows)

	1	2	3	4	Total
1	26	16	–	3	45
2	2	2	1	–	5
3	3	5	1	1	10
4	8	1	–	2	11
5	1	1	–	–	2
Total	40	25	2	6	73

Examination II (1962)

ES-group (columns); S-T T group (rows)

	1	2	3	4	5	Total
1	19	9	2	3	1	34
2	1	2	1	–	3	7
3	3	1	–	–	1	5
4	6	–	1	–	1	8
5	10	6	1	2	–	19
Total	39	18	5	5	6	73

these only 2 and 4 respectively had these arrhythmias only at rest

In the exercise ECG records there were at examination I 33 and at examination II 34 (bout 45 %) subjects who had ES or an ectopic atrial rhythm (for details see table IV) Of these, 27 and 26 respectively had the arrhythmia only on exercise. In these respects there was no difference between the two blood pressure groups. Altogether 48 (66 %) subjects had ES at one or both examinations.

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ECG at rest. Table VI shows how the individual S-T T items changed between examination I and II Among the subjects with a *normal blood pressure* at examination I there was one subject with group 2 and one with group 5 S-T T

Table III Frequency of various symptoms A—F at examination I and II The underlined numbers refer to subjects with high blood pressure

Examination I

Examination II	No symptoms	A	B	C	D	E	Total
No symptoms	31 12	— 2	2 1	— —	— —	— —	33 15
A	1 —	— —	— —	— —	— —	— —	1 0
B	— 1	2 —	— —	— —	— —	— —	2 1
C	2 3	— 2	1 —	1 1	— —	— —	4 6
D	1 1	— 1	— —	— —	— 1	— —	1 3
E	— 2	— —	— —	— —	— —	— 1	0 3
F	— 3	— 1	— —	— —	— —	— —	0 4
Total	33 22	2 6	3 1	1 1	0 1	0 1	41 32

panied by changes in the S-T segment Lepeschkin has stressed (10) that the S-T changes reveal more information about the coronary blood flow than the T changes do. To simplify the presentation of the results, the T wave changes have been included under code IV *groups I—5* together with the S-T changes.

The maximum degree of arrhythmia at rest, at any level of work or at any occasion post exercise was coded The code was simplified and the arrhythmias grouped as 2 3 4 or 5 (table II)

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Four subjects died during the follow up period one of congestive heart failure which was already manifest at examination I two of bronchial carcinoma, one of myocardial infarction. This last subject had high blood pressure and was over weight and he had symptoms of type D but neither S-T T changes nor ES in the ECG from examination I

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a Blood pressure and body weight

At examination I 46 subjects had normal blood pressure and 27 had high blood pressure at examination II the corresponding numbers were 41 and 32 respectively Five subjects had border line blood pressures at examination I and high blood pressures at examination II. In the tables where the two groups of subjects with normal and high blood pressures respectively are compared at the two examinations, these 5 subjects are classified as having high blood pressures at both occasions.

Symptoms were significantly more frequent in the group of subjects with high blood pressure ($0.01 > P > 0.001$ see table III)

The frequency of high blood pressure is higher in the group with overweight than in the group of normal or under weight subjects. The difference is statistically probably significant ($0.05 > P > 0.01$)

b ECG changes — symptoms

Arrhythmias (Code IX, table II) At rest at examination I 3 persons had VES (ventricular ectopic beats) 4 had SVES (supraventricular ectopic beats) and 1 had an ectopic atrial rhythm. At examination II 3 had VES 7 had SVES and 1 had both VES and SVES Of

1—5 was observed from examination I to II

Exercise ECG On exercise 8 subjects with *normal blood pressure* at examination I had S-T T changes which were coded as groups 3—5 (table VII) Of these 8 subjects 6 had more severe S-T T changes at examination II while 1 in group 3 did not change and 1 remained in group 5.

Of the 32 persons with a *high blood pressure* there were 15 with S-T T changes of type 3—5 at examination I Of these 15 subjects 2 had smaller S-T T changes at examination II than t examination I, 4 had the same and 9 had more severe changes.

Fifty subjects were coded to S-T T groups 1—2 t examination I of these 10 had developed changes of type 3—5 at examination II. The symptoms are given in table VII.

T sum up the results from the exercise ECGs At examination I 45 subjects (61 %) had no S-T T changes, 5 had S-T T changes of group 2 (7 %) 10 group 3 (14 %) 11 group 4 (15 %) and 2 group 5 (3 %) At examination II the corresponding numbers were 34 in group 1 (46 %) 7 in group 2 (10 %) 5 in group 3 (7) 8 in group 4 (11 %) and 19 in group 5 (26 %)

Subjects with high blood pressures manifested more S-T T changes in the resting as well as in the exercise ECGs than did subjects with normal blood pressures. They had also symptoms more frequently In 20 % of the whole material a change in exercise ECG from groups 1—2 to groups 3—5 was observed from examination I to II Of 21 subjects who had an exercise ECG with groups 3—4 S-T T changes at examination I 12 (57 %) were classified as group 5 t examination II. Two subjects with changes

Table VII Frequency of S-T T changes of different severity groups in exercise ECG at examination I and II and the frequency of various symptoms by S-T T changes

Subjects with normal B. P

Examination I

Examination II: S-T T items group	1	2	3	4	5	Total
1	23 O-A A-B B-O	—	—	—	—	23
2	2	2	—	—	—	4
3	1 B-C	—	1 O-D	—	—	2
4	1	—	2 B-O	—	—	3
5	2 O-C O-C	—	1 A-B	3 C-C	1	7
Total	31	2	4	3	1	41
Subjects with high B. P						
1	9 A-O O-D O-F	—	—	—	—	9
2	1	1	—	1 A-D	—	3
3	1	—	1 O-F	1 B-O	—	3
4	1 A-C	1	1 O-B	2 A-O D-D	—	5
5	2 C-C O-E	1 A-C	4 O-C O-C	4 A-F O-E E-E O-F	1 O-O	12
Total	14	3	6	8	1	32

Legend of the letters see table VI

of type 5 remained in this group at examination II.

Only 1 of 16 subjects *with symptoms* at examination I had S-T T changes at *rest* classified in a group higher than 2. At examination II the corresponding

Table VI Frequency of S-T T changes of different severity groups in rest ECG at examination I and II, and the frequency of various symptoms by S-T T changes Each letter combination e g A-B represents the type of symptom in one subject The first letter refers to the symptom at examination I the second is the symptom at examination II Only subjects with symptoms at at least one examination are listed

Subjects with normal B. P

Examination II: S-T T items groups	Examination I: 1	2	3	4	5	Total
1	34 A-B, O-A A-B, O-C B-C, B-O	—	—	—	—	34
	—	—	—	—	—	—
3	4 C-C, B-O O-D	—	—	—	—	4
4	—	1	—	—	—	1
5	1 O-C	—	—	—	1	2
Total	39	1	—	—	1	41

Subjects with high B. P

Examination II: S-T T items groups	Examination I: 1	2	3	4	5	Total
1	20 A-C, D-D A-D O-C A-O O-D A-O O-F B-O O-F	—	—	—	—	20
2	—	—	—	—	—	—
3	5 A-C, O-C O-B, O-F	—	—	—	—	5
4	2 O-E A-F	—	2 O-C	—	—	4
5	2 C-C O-E	—	1 E-E	—	—	3
Total	29	—	3	—	—	32

A = palpitations or awareness of the heart at rest.
B = chest symptoms other than A and C at rest and/or during work.
C = angina pectoris.
D = dyspnea during work that had increased during the last year.
E = symptoms and/or signs of cardiac decompensation.
F = was receiving or had received benefit payments for some time during the last two years because of symptoms of high blood pressure.
O = free from symptoms.

changes. These two ECG.s were coded as group 4 and 5 respectively at examination II (one probably had had scarlet fever myocarditis and the other rheumatic fever) None of them had symptoms on either occasion.

In the group of subjects with *high blood pressure* there were 3 codified with ECG changes group 3 at examination I Two of these subjects had ECG changes group 4 and one had ECG changes group 5 at examination II (table VI)

Fifteen subjects in S T T groups 1—2 at examination I were classified as groups 3—5 at examination II (4 with high amplitude R waves of the left type, 1 with a ventricular activation time of 0 06 sec. or more, 3 with both these changes) The symptoms are given in table VI

To summarize 68 subjects at examination I had resting ECG.s classified as group 1 (93 %) 1 as group 2 (1 %) 3 as group 3 (5 %) none as group 4 and 1 as group 5 (1 %) At examination II the corresponding numbers were 54 (74 %) none, 9 (12 %) 5 (7 %) and 5 (7 %) In 21 % of the whole material a change from groups 1—2 to groups

3—5 was observed from examination I to II

Exercise ECG On exercise 8 subjects with *normal blood pressure* at examination I had S-T T changes which were coded as groups 3—5 (table VII) Of these 8 subjects 6 had more severe S-T T changes at examination II while 1 in group 3 did not change and 1 remained in group 5

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T sum up the results from the exercise ECGs At examination I 45 subjects (61 %) had no S-T T changes, 5 had S-T T changes of group 2 (7 %) 10 group 3 (14 %) 11 group 4 (15 %) and 2 group 5 (3 %) At examination II the corresponding numbers were 34 in group 1 (46 %) 7 in group 2 (10 %) 5 in group 3 (7 %) 8 in group 4 (11 %) and 19 in group 5 (26 %)

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Table VII Frequency of S-T T changes of different severity groups in exercise ECG at examination I and II and the frequency of various symptoms by S-T T changes

Subjects with normal B. P

Examination I

S-T T items group	1	2	3	4	5	Total
1	25 O-A A-B B-O	—	—	—	—	25
2	2	2	—	—	—	4
3	1 B-O	—	1 O-D	—	—	2
4	1	—	2 B-O	—	—	3
5	2 O-C O-C	—	1 A-B	3 C-O	1	7
Total	31	2	4	3	1	41

Examination II

Subjects with high B. P

S-T T items group	1	2	3	4	5	Total
1	9 A-O O-D O-F	—	—	—	—	9
2	1	1	—	1 A-D	—	3
3	1	—	1 O-F	1 B-O	—	3
4	1 A-C	1	1 O-B	2 A-O D-D	—	5
5	2 O-C O-E	1 A-C	4 O-O O-O	4 A-F O-E E-E O-F	1 O-C	12
Total	14	3	6	8	1	32

Legend of the letters see table VI

of type 5 remained in this group at examination II

Only 1 of 16 subjects *with* symptoms at examination I had S-T T changes at *rest* classified in a group higher than 2. At examination II the corresponding

Table VIII Frequency of S-T depressions at rest during and after exercise at examination I and II
For classification see table II

S-T depression class	Rest	Examination I			Rest	Examination II		
		During highest load	1 min after	3 min after		During highest load	1 min after	3 min after
No depr	64	27	42	35	45	15	29	20
IV 1	1	3	2	2	5	16	8	19
2	—	3	2	8	4	3	5	6
3	—	—	—	3	1	—	1	2
4	3	—	1	10	9	—	[illegible]	5
5	1	32	13	5	—	31	7	7
6	4	8	13	10	9	8	21	14
Total	73	73	73	73	73	73	73	73

Table IX Correlation between symptoms by history and symptoms at the exercise test

Examination I (1957)

Symptoms during the exercise test	Symptoms from the medical history					
	A	B	C	D	E	Total
Dyspnea	5	—	1	—	1	7
Angina pectoris	—	—	—	—	—	0
General fatigue or leg exertion	1	4	1	1	—	7
No complaints	2	—	—	—	—	2
Total	8	4		1	1	16

Examination II (1962)

Symptoms during the exercise test	Symptoms from the medical history						
	A	B	C	D	E	F	Total
Dyspnea	1	—	2	4	2	1	10
Angina pectoris	—	—	5	—	—	—	5
General fatigue or leg exertion	—	2	—	—	—	2	4
No complaints	—	1	3	—	1	1	6
Total	1	3	10	4	3	4	25

number was 13 out of 25. In the exercise ECG records the corresponding numbers were 9 out of 16 at examination I and 20 out of 25 at examination II. This difference between rest and exercise ECG:s and their correlation to symptoms is statistically significant at both examinations ($0.01 > P > 0.001$ and $0.02 > P > 0.01$ respectively).

In this connection it is of interest to

know how many subjects there were who showed S-T T changes in their exercise ECG records but did *not* have any symptoms. At examination I 24 men had group 3—5 S-T T changes in the ECGs. Fifteen of them had no symptoms. Of these 15, however 8 had symptoms at examination II. On the other hand, 3 of those *with* symptoms at examination I were free from symptoms at the re-examination. Of these 3, two had however high blood pressures which had improved and the third person had changed to a physically less strenuous occupation. Thus in principle the subjects who had S-T T changes in their exercise ECG records at examination I, had more severe S-T T changes at examination II The majority of those who were symptom free at examination I had eventually acquired symptoms (table VII)

S-T junctional depressions of 0 6 mm or more, with a normal shape of the S-T segment, appeared during exercise in 53 % at examination II (table VIII) The average heart rate was 1₀3 n 66 subjects when the exercise was stopped. The correlation between these changes and symptoms was low

Angina pectoris. A t tal number of 10 subjects had a history of angina pectoris (?) at examination II Of these, 5 had symptoms already t examination I Four of these 10 men had had one or more ECG-verified myocardial infarctions between the two examinations (2 with a normal blood pressure and 2 with high blood pressures) All had symptoms at examination I (1 (A) 1 (B) and 2 (C)) but none of these 4 had S-T T changes of a higher degree than type 2, neither in the resting nor in the exercise records. Three had occasional ES

At examination II termination of the exercise test was motivated by angina pectoris in 5 of the 10 subjects mentioned above (S-T T changes of group 5 in all 5) In 3 with a history of myocardial infarction the test was stopped before any symptoms appeared. The remaining 2 complained of dyspnea during work.

Table IX lists the symptoms obtained from the medical history as compared to those experienced during the bicycle test on both occasions.

c Blood lactic acid concentration and heart rate

On a work load of 600 kpm/min., corresponding to an oxygen uptake of about 1.5 l/min. 66 subjects had a heart rate of 116 ($1 \times \sigma = \pm 15$) at examination I and 121 ($1 \times \sigma = \pm 16$) beats/min. at examination II. The remaining 7 persons had a definitely reduced capacity for physical work at examination II They could perform work only at a load of 300 kpm/min., 3 because of angina pectoris.

At the highest load for these 66 subjects a mean blood lactic acid concentration of 5.2 mEq/l (57 mg $1 \times \sigma = \pm 14$) was reached at examination I compared to 4 7 mEq/l (52 mg $1 \times \sigma = \pm 14$) at examination II A greater difference was expected as an attempt was made to reach a maximal load at examination I but not at examination II

Discussion

This material consists of a highly selected group of men engaged in a specific occupation. Thus, the demonstrated frequency of coronary heart disease cannot be taken as representative of the male population of Stockholm.

In this material there is a statistically significant higher correlation between symptoms and ECG findings on exercise than during rest. These results strongly support the validity of the exercise ECG

changes as classified in the code Similar strong correlations to exercise ECG changes have been found in studies, where patients with angina pectoris have been compared to clinically healthy controls (2 6 13)

The strictly junctional type of S-T depressions may represent a normal change in the repolarization pattern with increasing work loads and heart rate. A junctional depression appearing at a low heart rate may be of significance whereas a similar change at a high heart rate should be considered entirely normal.

Five men in the original material of 81 subjects developed a myocardial infarction between the two examinations (2 with normal body weight and normal blood pressure, 1 with normal body weight and high blood pressure, 2 with overweight and high blood pressure) None of these had S-T T changes of a degree higher than that appropriate for classification in group 2 at examination I The fact that a myocardial infarction thus occurred in these 5 men but in none of those with severe S-T T changes at examination I reflects the highly variable course of coronary heart disease. In a given case there may be a slow progressive sclerosis of the vessels, in another a more rapid development of the disease leading to a sudden occlusion of an artery In the first condition there may be time for the development of collateral blood vessels while in the second an injury causing permanent damage may occur before a collateral circulation can be established

From the results it is evident that the appearance of ES in connection with exercise may be incidental. It is also obvious that ES often differ in character from time to time The poor correlation between the presence of ES and the degree of S-T T changes suggests that ES may be of a different etiology

It has often been stressed in the literature that the appearance of ES on exercise is a sign of coronary heart disease (12) Sandberg (16) states this to be valid only for elderly persons. Other authors, e. g Lepeschkin and Surawicz (11) are of an opposite opinion. The present results agree with the latter i e. that the appearance of ES has little to do with coronary heart disease.

Both S-T T depressions and appearance of ES might however be a sign of an ageing process which may not truly connote actual disease. In a material of healthy young subjects up to an age of about 40 years the frequency of S-T T changes or of ES is markedly lower (3, 25) There is no reason to believe that heavy daily work, even if it is of such a special kind as the distribution of beer should give rise to any changes (9 a. o.)

Subjects with a well defined history of angina pectoris when walking might be expected also to experience symptoms when cycling at least when the exercise is followed by S-T T changes of group 5 In this material only about 50 % of such subjects complained of angina pectoris, even at loads higher than what corresponds to their daily work. This relationship might depend upon the two different types of physical strain.

After a re-examination it is of interest to correlate the objective findings at the first examination with the symptoms at the second in other words to analyse if it could have been possible with any method to anticipate the development of clinical symptoms. Such an analysis gives the interesting result that of the methods employed in this study the recording of exercise electrocardiograms yields most information in this respect.

Summary

1 Seventy three men 35 to 70 years old were re-examined after an initial examination 5 years ago Both examinations included ECG recording at rest, during and after exercise The physical work was performed on a bicycle ergometer at stepwise increased work loads. A 16-lead ECG (with precordial V and CR-leads) was recorded at rest, leads CH_5, CH_4, CH and CH were recorded during work. ECG changes were classified according to a modification of the Minnesota code (5)

Rest and exercise S-T T changes were summarized by means of a 5-grade severity code.

2. Sixty-six per cent of the subjects had ectopic beats (ES) at one or both examinations. There was no correlation between the degree of S-T T depression and the frequency of ES in the exercise ECG records.

3 In 20 per cent of all subjects a change in the classified S-T T items from severity groups 1—2 to severity groups 3—5 was observed in the exercise ECGs between examination I (1957) and II (1962) and in 37 per cent from severity groups 3—4 S-T T changes to group 5

4 The correlation between chest symptoms by history and S-T T changes was significantly higher when recorded during and after exercise than when recorded during rest.

5 The significance of the strictly junctional type of S-T depressions is discussed It is concluded that the shape of the S-T segment should always be considered when interpreting the results of an exercise test.

6. Five subjects developed a myocardial infarction between the two examinations. All had only minor exercise S-T T changes at examination I

7 Only half of the subjects with a history of angina pectoris had anginal symptoms during the cycle ergometer test but all of them had typical ischemic S-T T changes.

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Addendum

Since the manuscript was prepared Thomas W Mattingly published a paper with the title "The postexercise electrocardiogram in Amer. J. Cardiol. IX 395, 1962. Results of the present study are in applicable parts in agreement with his observations.

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From the Medical Clinic I (Head L. Werkö, M. D.) and the Roentgendiagnostic Clinic I (Head S. R. Kjellberg, M. D.) Sahlgren Hospital, University of Gothenburg, Sweden

Coronary Angiography in the Diagnosis of Coronary Heart Disease

By

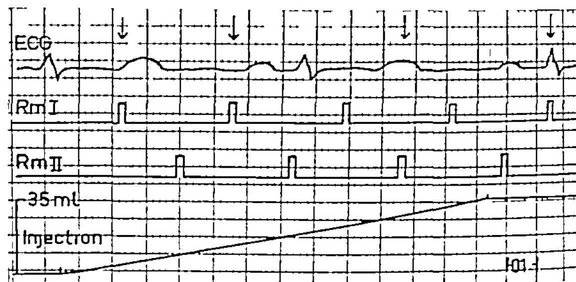
S. A. Forsberg, S. Paulin, E. Varnauskas and L. Werkö

The great interest shown in coronary disease during recent years has been evidenced mainly by extensive population studies, in which attempts have been made to determine the significance of different environmental factors in the development of the disease. Comparisons of morbidity and mortality and certain relatively accessible environmental factors such as diet, fat consumption, type of employment, and amount of physical exertion, have been carried out in a great many countries on populations of varying magnitude. These investigations have not succeeded in defining the pathophysiological mechanisms which govern the correlated results. They have provided a stimulus, however, to the development of new subjects of inquiry within the clinical and experimental study of coronary disease.

In the majority of studies, sudden death was taken as indicating the presence of coronary disease or cardiac infarction. In a recent publication from Westchester County New York (15) an analysis was made of autopsy findings in cases of unexpected sudden death. Only in those men in whom death occurred within one hour of the onset of the fatal illness was coronary disease or cardiac infarction the cause of death in as many as 90 of cases. In women, or where a longer time had elapsed after the onset of illness, the incidence of cardiac infarction was less than 60 %.

Despite the fact that certain therapeutic advances have been made, the treatment of coronary disease, and particularly its final stage, cardiac infarction, is relatively unrewarding. In the absence of effective prophylactic measures against arteriosclerosis or coronary thrombosis, in the entire population, interest must be concentrated in an attempt at early screening of those groups of patients who can be said, on the basis of statistics, to run a great risk of developing coronary disease. American studies have shown convincingly that at least in American material

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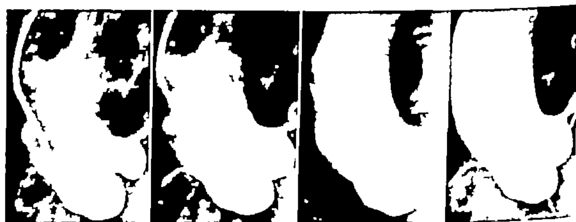


Fig. 1 a—d.

Fig 1 Shows details of the aortic bulb during contrast injection. The arrows indicate the moment exposure of the four pictures in relation to the ECG Rm I and Rm II are the exposure markings of the two alternating X ray tubes in stereoscopic projection. 35 ml 76 Urografin was injected. The distance between two coarse time lines is equal to 0 1 sec.

there is a distinct correlation between an increased mortality due to cardiac in farction in men between 45 and 60 years of age and a raised blood cholesterol level raised blood pressure and obesity (3 8, 16) It is as yet unknown whether the prognosis can be favourably influenced by correction of any of these factors. Moreover we do not know whether these data are relevant for Swedish material. In fact in a recent WHO classification Sweden was grouped with Italy Portugal and Japan etc. with a low mortality due to cardiosclerosis, in spite of the high fat consumption and high serum cholesterol which are to be noted in Sweden.

The present investigation forms part of a larger study aimed at *following* a smaller population with coronary heart disease or increased risk of developing coronary occlusion. More exact study of the anatomy of the coronary arteries, metabolic factors of importance and the influences of the stresses of daily life in

a smaller and more defined group is felt to contribute more information to the important question of the etiology of coronary occlusion than extensive comparisons in larger populations.

The diagnosis of coronary disease is made on the basis of ECG changes and/or the history of pain, and in many cases of recent cardiac infarction with the further support of certain laboratory tests. Even though a good correlation exists between these criteria and coronary disease, it must be borne in mind that the clinical picture is the result of myocardial hypoxia, perhaps with necrosis, but provides no direct evidence of the state of the coronary arteries. It is therefore a matter of the utmost importance to reach a method of investigation by which the anatomy and pathology of the coronary arteries in vivo can be brought to light. With this aim in mind we have taken up coronary angiography

Before coronary angiography can be accepted as an adequate examination of the anatomy of the coronary vessels, a number of questions require to be answered. Can the risk of complications in connection with coronary angiography be maintained so low that the investigation may be performed on fairly wide indications Can the technique of the method be simplified to a procedure so simple, for both patient and examiner that it can be employed as routine, even for differential diagnosis Can angiography provide a complete and reliable picture of the coronary arteries What types of changes in the vessels can be diagnosed in this way This last question cannot be answered without correlative studies of the pathological anatomy symptomatology and ECG changes in groups of patients examined by coronary angiography



Fig. 2. Detail from coronary angiogram in 28-year-old man. A is taken during diastole while the contrast is being injected, and the whole of the bulb of the aorta, and the coronary arteries, are well filled. B is also taken during diastole but immediately after completing the injection. Sedimentation of contrast can be clearly seen in the right coronary artery

In our choice of the coronary angiographic technique, these problems have been taken into consideration.

Roentgen technique

The foremost requirement for an assessable coronary angiogram is that a sufficient amount of contrast material is introduced into the coronary circulation. Simultaneous filling of the heart chambers with contrast should be avoided, since this obscures the coronary vessels and makes proper examination of them impossible. The contrast should therefore be injected into the ascending aorta, whatever the type of catheter or technique of puncture. A number of factors can hinder contrast filling of the coronary vessels. The big blood reservoir of the ascending aorta leads to dilution of the

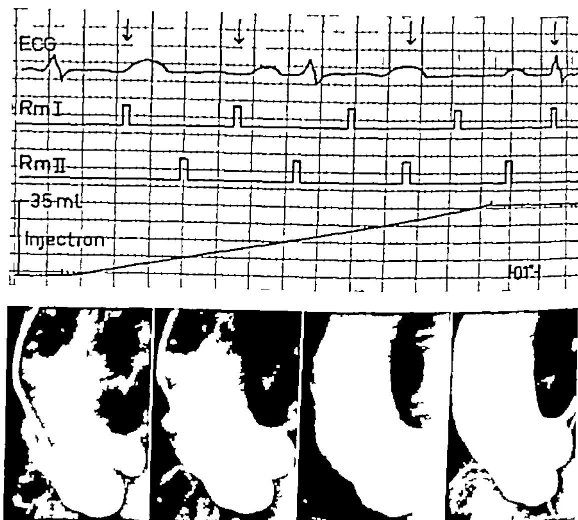


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contrast material. In addition there may be uneven spreading of contrast in the ascending aorta as a result of the position of the catheter or the puncture needle, and in turn, one or other of the coronary arteries may be filled poorly or not at all (7). Different means have been attempted to ensure the adequate introduction of contrast into the coronary arteries (6, 1, 4, 10, 14). The technique which we employ is based on that described by Olin (12) and by Bellman et al. (2) in which the tip of the catheter is twisted to form a ring. We use a new thin-walled radio-opaque catheter (Kifa) which permits the high injection rate of more than 30 ml 76 % Urografin per second, so important for the success of the examination. The catheter is introduced via one of the femoral arteries, according to the method described by Seldinger. The ring-shaped portion of the catheter is placed in the ascending aorta, as near as possible to the aortic valves. Through the lateral perforations in the catheter on a level with the ring the entire bulb of the aorta together with the orifices of the coronary arteries can be filled virtually instantaneously. Since blood from the left ventricle courses, for the most part, through the ring portion of the catheter and the jets of contrast are directed towards the periphery, the coronary vessels are supplied with contrast during both systole and diastole (fig. 1).

A very important detail of the method is that the examination must be performed with horizontal X-ray beam, so that any sedimentation of contrast (9) may be assessed (fig. 2).

We have carried out a detailed study of the catheter technique described here. The significance of the injection of contrast in the different phases of the cardiac cycle has been examined, as well as the sedimentation of contrast, the positioning of the patient, and different factors of exposure. We have compared the contrast filling of the coronary arteries during systole and diastole, and studied the effect of alterations in blood pressure or pulse rate, and the effects of different drugs. The results thus obtained are to be published and discussed elsewhere (13).

We use a stereoscopic technique (11) with 2 alternating X-ray tubes and perform 2 injections, one with the patient in a lateral and one with the patient in a left anterior oblique position. Each time 30—50 ml 76 % Urografin is injected. In the case of coronary vessels which radiologically appear normal, a satisfactory result could be achieved with much smaller amounts of contrast. In our experience, however, the presence of advanced stenosis or occlusion necessitated a duration of injection greater than one second, since the circulation of contrast is slowed down in the affected blood vessel.

Preparation of the patient for the examination involves only premedication with morphine and scopolamine. No anesthesia is given.

To date, we have performed 140 coronary angiographies by the method described here. No serious complications have occurred. In one patient, who had a narrow aortic stenosis, the injection of a small trial dose of contrast was followed by a severe drop in blood pressure, with an associated ventricular tachycardia. The examination in this case was abandoned. In five patients catheterization of the ascending aorta was rendered impossible by tortuous, arteriosclerotic pelvic arteries.

The patients were strikingly free from discomfort during the injection of contrast and admitted only a slight to moderate sensation of warmth. In four patients, the examination was repeated after about a week's interval, on account of a technically poor result on the first occasion.

The pressure in the aorta is measured at regular intervals during the examination. In more than half of our cases, the aortic pressure was measured immediately before and after the injection of contrast or simultaneously during the whole procedure. Immediately following injection, a slight drop in blood pressure has often been registered, together with a slight tachycardia, both lasting, on an average, about 1—2 min. In a few cases, the ECG has shown slight ST—T-changes, and in a very few instances, the patient complained of slight anginal discomfort during the examination. This pain did not appear to be directly related to the contrast injection, but seemed rather to be the result of the examination procedure. It was noted only in patients who had a previous history of angina pectoris.

Material

An account is given here of some preliminary results which have been obtained by studying the collected clinical data of 40 of our first 44

Table I

Age	♂	♀	Total
< 40	9	0	9
40–49	12	2	14
50–59	11	4	15
> 60	0	2	2
Total	32	8	40

coronary angiographic cases. Four cases have been excluded because the films obtained were of inferior quality. The clinical diagnoses were made prior to coronary angiography and were therefore independent of the results of this examination. Table I shows how the clinical material is comprised with regard to age and sex of the patients. There were various indications for performing coronary angiography. In many cases the diagnosis of coronary disease was probable prior to angiography, whereas in some cases it was dubious. In a final group of patients, there was no suspicion of coronary disease, to judge from the history and ECG finding; this was the case in certain patients with hypercholesterolaemia and others with the diagnosis cardiac neurosis.

Regard was taken to the type of pain in the history and the ECG findings[1].

The term coronary pain (angina pectoris) is used here to describe recurrent and transient pain, localized to the praecordium or retrosternal region, and related to exertion. Pain unrelated to exertion, or atypical in its long duration, or pain situated atypically in the epigastric region, thorax, dorsal spine, neck, shoulders or arms, is described here as "partly contra-indicating coronary pain (angina pectoris)". The absence of relationship to exertion, in combination with atypical localization of pain or the complete absence of pain, was accepted as "not characteristic of angina pectoris". The group described as having typical coronary pain also includes those with pain of infarction type, i.e. steady, severe pain, retrosternal or praecordial in localization, and estimated as persisting for an hour or more.

Almost all ECG-s were carried out in the Department of Clinical Physiology.

Table II

		Angio with obstr	Angio without obstr
Typical angina pectoris Previous cardiac infarction ECG with pathol. Q	}	5	0
Typical angina pectoris Previous cardiac infarction ECG with pathol. ST and/or T	}	3	1
Typical angina pectoris No history of cardiac infarction ECG with pathol. ST and/or T	}	5	5

Results

All patients with the clinical criteria of coronary disease are collected in table II. The first group comprises patients with at least some period of typical angina pectoris, a history of previous cardiac infarction diagnosed in hospital, and an ECG with pathological Q-waves, the latter persistent in all cases. The five patients who fulfilled these criteria all showed obstruction in the coronary arteries, on angiography. The second group comprises those patients with a period of typical angina pectoris in the history, previous infarction diagnosed in hospital, and having at some period shown pathological ST or T-changes in the ECG. In one of these four no obstruction of the coronary arteries was made apparent by angiography. The patient was a man who had been admitted to hospital under the diagnosis cardiac infarction about three years previous to angiography. Scrutiny of the case report from this occasion revealed that the diagnosis pulmonary infarction must be strongly suspected. In an early phase of his illness a considerable consolidation was demonstrated in the lower lobe of one lung, at

contrast material. In addition there may be uneven spreading of contrast in the ascending aorta as a result of the position of the catheter or the puncture needle and in turn one or other of the coronary arteries may be filled poorly or not at all (7) Different means have been attempted to ensure the adequate introduction of contrast into the coronary arteries (6 1 4 10 14) The technique which we employ is based on that described by Olin (12) and by Bellman et al (2) in which the tip of the catheter is twisted to form a ring We use a new thin walled, radio-opaque catheter (Kifa) which permits the high injection rate of more than 30 ml 76 % Urografin per second, so important for the success of the examination. The catheter is introduced via one of the femoral arteries, according to the method described by Seldinger The ring shaped portion of the catheter is placed in the ascending aorta, as near as possible to the aortic valves. Through the lateral perforations in the catheter on a level with the ring the entire bulb of the aorta together with the orifices of the coronary arteries can be filled virtually instantaneously Since blood from the left ventricle courses, for the most part, through the ring portion of the catheter and the jets of contrast are directed towards the periphery the coronary vessels are supplied with contrast during both systole and diastole (fig 1)

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Material

An account is given here of some preliminary results which have been obtained by studying the collected clinical data of 40 of our first 44

of whom had only one episode with pain of infarction type and 1 of whom had an episode of infarction pain besides pains of a type partly contradicting angina pectoris. Eight of these patients had no visible obstruction in the coronary arteries. The diagnoses in these cases were as follows

Hypertension + obstructive pulmonary emphysema	1
Hypertension	2
Myocarditis	1
Aortic stenosis	1
Chronic bronchitis	1
Pericarditis	2

The cases include all those six lacking visible obstruction in table II and, more over one case with acute pericarditis and another who had previously had acute pericarditis. In seven out of these eight cases we thus found clinical diagnoses which are compatible with pains of coronary type in the absence of obstructions in the larger coronary arteries.

The second group in table III comprises patients with a pain syndrome earlier characterised as partly contra dicting coronary pain (angina pectoris) Both patients, who had demonstrable obstruction, had pathological ST and/or T-changes in the ECG t rest without any extracoronary causes of a pathological ECG-reaction.

Of the seven who had no clinical suspicion of coronary disease none showed evidence of obstruction on coronary angiography

Finally in table IV ECG changes are compared with angiographic findings. All patients hav been examined during their stay at hospital using the standard leads, the unipolar chest leads (CR1 CR2, CR4 CR5 CR7) These leads have been used as a routine at the Dept. of Clinical Physiology since 1955 In many patients the ECG has been followed with repeated examination over a period of years. The ECG on exertion was obtained in all but 7 patients, and of these 6 had a pathological resting ECG Regard has been taken to treatment w th digitalis in estimating the ECG changes.

Those 2 patients who in spite of normal ECG at rest and on exertion, showed evidence of obstruction on angiography had both a history of typical angina pectoris.

Three patients with no signs of obstruction on angiography had nevertheless pathological ECG changes on exertion (resting ECG normal) One of these (the patient earlier described as having the diagnosis chronic bronchitis) had pain of coronary type, the cause of which was never revealed. The two others had no angina pectoris, but one of them had hypercholesterolaemia.

In twelve patients no obstruction was noted on angiography despite pathological ST and/or T-changes even at rest. The clinical diagnoses here were as follows

Hypertension	2
Hypertension + obstructive emphysema	1
Myocarditis or pericarditis	3
Aortic stenosis	2
I termittent left branch block	1
Right branch block + inactive pulm. T.B.	1
Bronchiectasis	1
Cardiac neurosis	1

In the two patients with aortic stenosis, it is possible that digitalis may have influenced the ECG Similarly the patient with the diagnosis of cardiac neurosis

Table III

Type of pain	Angio with obstr	Angio without obstr	Total
Typical coronary pain	15	8	23
Partly contraindicating coronary pain	2	8	10
Contraindicating coronary pain	0	7	7
Total	17	23	40

Table IV

ECG	Angio with obstr	Angio without obstr.	Total
Normal	2	7	9
Pathol. ST and/or T only on exertion	2	3	5
Pathol. ST and/or T at rest	8	12	20
Pathol. Q	5	1	6
Total	17	23	40

the time when the patient complained of pleuritic pain and ECG showed transient pathological ST—T-changes of a type as seen also in pulmonary infarction Other observations included lung emphysema of obstructive type, and a moderate hypertension which may explain the slight ST—T-changes seen at the time of angiography

The final group in table II comprises patients with a period of typical angina pectoris in the history no previous hospital diagnosis of cardiac infarction but who at some period had pathological ST and/or T-changes in the ECG during rest or at work test In five out of ten patients there was no evidence of obstruction on angiography The clinical diagnoses in these five cases were as follows

Hypertension	2
Myocarditis	1
Aortic stenosis	1
Chronic bronchitis	1

Both patients with hypertension had several years history of high blood pressure, retinal changes corresponding to fundus hypertonicus grade II and signs of left ventricular enlargement. One of them had a considerable degree of cardiac failure. The patient with myocarditis was a 38-year-old man who became ill 3 years previous to the performance of coronary angiography with a febrile throat infection which was followed by rapidly progressing heart failure. The patient diagnosed as aortic stenosis had signs suggestive of a moderate degree of stenosis. In the case with chronic bronchitis, no cause could be found for pain other than the suspicion of coronary disease. The resting ECG was normal, but on exertion a transient left branch block was noted

Thus, of the five cases in which coronary angiography showed no evidence of obstruction four had clinical diagnoses compatible with pain of angina type, and pathological ST and/or T-changes, in spite of the absence of obstruction in the larger branches of the coronary vessels.

Table III provides an analysis of the type of pain together with angiographic findings. A special pain history has been taken by the same examiner in all but two cases. The objection can always be raised that a pain analysis is based on subjective assessment by the patient and the examiner The first group in table III comprises 23 patients with pain described as typical coronary pain, 21 of whom had at least some period of angina pectoris, 1

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Cardiac neurosis	1

In the two patients with aortic stenosis, it is possible that digitalis may have influenced the ECG Similarly the patient with the diagnosis of "cardiac neurosis"



Fig 3 Coronary angiography (case 1) A. Oblique projection, the direction of the beam being parallel to the interventricular septum. The exposure occurs just before completion of the injection of 40 ml 76 % Urografin. B. The same case, lateral projection, this exposure being at the end of the second injection. The arterial distribution here is of the "right preponderance" type. The walls of the coronary arteries are even and smooth, and show no evidence of stenosis.

had somewhat inexplicably been given digitalis which may have produced the ECG findings noted The thoracic symptoms here were not in fact at all reminiscent of angina pectoris, and the patient gave the impression of neurosis. In the case with bronchiectasis, the ECG changes could not be explained Again the etiology in the two cases with bundle branch block was unknown neither of these, however had a history of typical coronary pain

Finally we found persistent pathological Q waves in a patient who nevertheless showed no evidence of obstruction on angiography He was a man about 38, who had been admitted to hospital about a year previously with an illness interpreted as perimyocarditis, beginning with severe pain of pleuritic type in the middle of the thorax, high fever with cardiac enlargement shown radiologically and a pericardial rub on auscultation. His ECG showed ST—T-changes of the type seen in pericarditis but in addition he developed pathological Q-waves. There was a moderate rise in the serum transaminase level (GOT) Prior to carrying out angiography it was suggested that the myocarditis had possible given rise to arteritis in a coronary artery with a resultant infarction In the light of the coronary angiography findings, however the question arises as to whether a myocarditis can primarily have caused so severe damage to the myocardium that pathological Q waves might result.

Report of three typical cases

Case 1 A woman, aged 51

Previous illness. The patient had previously had peptic ulcer with symptoms suggestive of ulcer in recurrent episodes and several attacks of colitis. Partial thyroidectomy was performed for thyrotoxicosis.

Present illness There had been a tendency to cough with expectoration, dating from childhood, but during recent years she noted occasional blood-stained sputa. During the past few years she had two episodes of pneumonia. Six years prior to coronary angiography there had been a chronic, recurring syndrome with chest pain of the type "partly contradicting angina pectoris" There had ever been periods of pain in the left arm.

Some laboratory results during hospital admission 1961—62 Physical examination of the heart was normal. Heart roentgen normal volume and configuration. B. P about 120/80. Resting ECG showed slightly abnormal ST—T changes over the ventral part of the left ventricl (CR2, CR4) and its lateral aspect (CR5 CR7) There was no significant difference in th ECG on exertion. Serum cholesterol and serum neutral f t were normal.

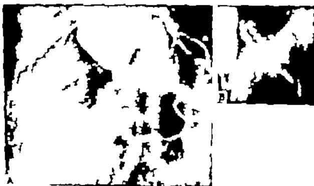


Fig. 4. Coronary angiography (case 2). A. Vessel distribution is of the "left preponderance" type. The posterior interventricular branch arises from the left coronary artery. There is severe narrowing over short extent of the left circumflex branch. In the main stem of the right coronary artery uneven calibre and irregularity of the vessel wall is noted. The large number of very fine arterial branches in the interventricular septum are evidence of collateral circulation. B. Detail illustration of the stenosed circumflex branch, in the lateral projection.



Fig. 5. Coronary angiography (case 3). A. Left preponderance. Unevenness of the vessel wall in virtually the whole extent of the left coronary artery. No contrast filling of the anterior interventricular branch, from the left coronary artery. Numerous, well-developed arterial branches in the interventricular septum suggest collateral circulation. B. Detail illustration about one second after the completion of contrast injection. The larger arteries are already emptied of contrast, while the anterior interventricular branch, and an intermediate branch to the left ventricle are still filled with contrast. With cine-film camera (48 exposures per sec) it was possible to demonstrate much more rapid contrast filling of the right coronary artery than of the left.

Lung roentgen. Marked changes in the lingula lobe, with densities in the parenchyma. Bronchography 3 years previously showed cylindrical bronchiectasis in the lingula. Cervical spine roentgen revealed degeneration of disc between fifth and sixth vertebral body.

Coronary angiography: see figs. 3 A and B.

Case 2. A man, aged 61.

Previous illness: Nothing of significance was noted.

Present illness: For three months previous to coronary angiography he had noted chronic, recurring chest pain, of gradually increasing severity and of the type described here as typical angina pectoris.

Some laboratory results during hospital admission 1961—62: Physical examination of the heart was negative. Heart roentgen showed normal size and configuration of the heart. B. P. 170/90. Optic fundi: "Fundus hypertonicus grade II". Resting ECG showed ST—T changes towards the diaphragm (II, III aVF) and over the lateral part of the left ventricle (CR5, CR7). Serum cholesterol — 281 227 mg %.

Coronary angiography: see figs. 4 A and B.

Case 3. A woman, aged 1

Previous history: She had had several episodes clinically suggestive of peptic ulcer. Duodenal ulcer was confirmed in the past, and the patient had also had several attacks of colitis.

Present illness: Hypercholesterolaemia was first confirmed 1958. The cholesterol level

varied between 250 and 380 mg with the patient on special diet. Xanthelasmata and tendon xanthoma was observed on general examination. 1958 she was admitted to hospital with cardiac infarction showing the typical clinical picture together with raised serum transaminase initially and a typical ECG. Since then, there has been increasing dyspnoea as well as a recurring chronic syndrome with chest pain of the type described as typical angina pectoris. Since 1960 she has belonged to the function group III—IV on account of coronary pain and dyspnoea. Towards the end of 1961 she developed diabetes mellitus.

Some laboratory results from hospital admission 1961—62. Physical examination of the heart: In the 3rd and 4th intercostal spaces to the left of the sternum, there was slight systolic pulsation and on auscultation an apical systolic murmur of grade II—III was heard. A 3rd sound could at times be distinguished in diastole. Roentgen showed normal size and configuration of the heart. The B.P. was about 120/80. ECG at rest showed pathological Q-waves in CR2, as seen after previous anterior wall infarction. In addition pathological ST—T-changes of varying degree were observed from time to time from the diaphragm at (aVF) and the ventral and lateral aspects of the left ventricle (CR4, CR5, CR7), but these may have been partly the result of digitalis. Serum cholesterol — 276 mg%. The patient's diabetes was well balanced.

Coronary angiography: see figs. 5 A and B.

Comments

By means of coronary angiography it is possible to demonstrate total occlusion and stenosis which lead to diminished calibre or unevenness of the wall in the larger arterial branches. It is often possible to appreciate slowing of flow in an obstructed artery and the secondary development of a collateral system, with the rapid flow of contrast in the other coronary artery. With cine film technique and a high exposure rate it was possible to assess the presence of rigidity in the walls of the larger arterial branches. I[...] this is at best a supplementary examin[...]tion in certain cases, since the 35 m[...] film camera with image intensifier c[...] only imperfectly reproduce detail.

Up to now we have in only one c[...] had the possibility of comparing the r[...]sults of coronary angiography with t[...] autopsy findings. The patient, a m[...] was found dead in bed at home, 14 d[...] after an uncomplicated coronary ang[...]graphy. At autopsy he was found to ha[...] no infarction, no coronary thrombosis[...] signs of recent damage at the orifices[...] the coronary arteries or in the arte[...] themselves. The coronary arteries w[...] however the site of grave, obstructive a[...]teriosclerotic changes. Angiography w[...] repeated post mortem and its result, [...]gether with the macroscopic appearan[...] of the coronary arteries when open[...] lengthwise at autopsy showed good agr[...]ment with the angiographic findin[...] during life.

The position of coronary angiograp[...] in clinical medicine of today can be su[...]marised as follows. It has been clea[...] shown that it is possible to carry [...] coronary angiography even in patie[...] with cardiac disease. Coronary ang[...]graphy is not yet to be regarded as [...] routine procedure. Lastly little has be[...] published regarding the relationship b[...]tween angiography findings and oth[...] clinical data. The preliminary resu[...] which we report here might suggest th[...] in certain cases coronary angiograp[...] can add important information for t[...] clinical diagnosis of coronary disea[...] It is to be expected that within the ne[...] future coronary angiography will be a[...]cepted as a valuable complement [...] present methods of investigation, and [...] great significance in differential diagnos[...] Coronary angiography is also likely

attain an important place in future studies of the various problems governing the etiology of coronary disease.

Summary

A preliminary analysis has been made of the coronary angiography findings in the first 40 cases examined in this way with a view to discovering whether coronary angiography can be an adequate clinical method of charting the anatomy of the coronary arteries.

The technique employed in coronary angiography is described. It is based, in principle, on the injection of contrast agent through a specially constructed catheter which is placed in the ascending aorta, as close as possible to the aortic valves. Roentgen films are taken at the rate of 6 per second. The investigation is made without anaesthesia and without any other pharmacological distortion.

The clinical material reviewed here includes patients with probable and doubtful coronary disease prior to examination, as well as a certain number in whom there was no indication of coronary disease previously.

An analysis has been made of the angiographic findings in relation to the ECG., type of symptoms and the clinical diagnoses made prior to the angiographic investigation.

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From the Medical Out Patient Clinic University Hospital, Copenhagen and the Protein Laboratory Institute of General Pathology Copenhagen, Denmark

A Case of α_2-Myelomatosis

By

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Presence of myeloma cells in the bone marrow is the most important finding for making a positive diagnosis of myelomatosis. Only when they have been found can the specific proteins be designated as myeloma proteins. Final confirmation of the diagnosis is given by the typical course of the disease when such a course is found, which does not always happen. The proteins can be characterized further by means of their properties with respect to electrophoresis, salting out and other procedures.

Most frequent are myeloma proteins which by electrophoresis show a migration rate within the β—γ-range whereas only a few instances have been described of unquestionable α_2-myelomas.

Sandkühler (6) has described one case of myelomatosis in which the abnormal protein on first analysis was found to be γ-globulin, but on a subsequent analysis had a migration rate like that of α-globulin. The Tiselius electrophoreses were shown solely on the positive side. However the pattern of this myeloma resembles that of an artefact rather than that of an α-myeloma. Kaipainen (4) found two cases of α myelomatosis, but the cases seem very doubtful. There are no illustrations of the results of electrophoretic analyses but only indications of the percentage distribution of the fractions. In addition to the high α_2-fraction both cases presented a raised γ-fraction, a finding which rather militates against a diagnosis of α-myelomatosis. The sternal marrow was not analysed.

Haarstad Larsen and Giertsen (1) found one case of α myelomatosis. In this case paper electrophoresis revealed an abnormally large fraction. Comparison with a reported electrophoresis of the albumin fraction after Howe-fractionation showed the myeloma protein to migrate at the same rate as α_1-globulin.

Wiedemann (7) described one case which by comparison with the other diagrams in the paper was found to be one of α-myelomatosis.

Wuhrmann and Wunderly (8) diagnosed 20 electrophoretically analysed cases as cases with α-myelomas. Of these no more than three seem convincing

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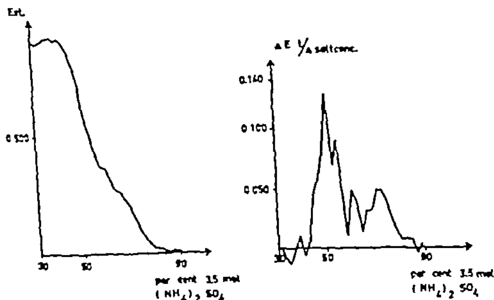


Fig. 2. Polyfractionation of serum according to Effersøe

β 12 ,, γ 9 1 2. The pattern highly suggests a_2-myeloma, possibly macroglobulin. This is a form of myelomatosis which I have not seen before. However ... few convincing cases have been described. (signed N. Harboe).

Salting-out fractionation according to Effersøe (fig. 2): A high narrow peak corresponding to 50 ... 3.5 mol. (NH ,SO = 100). "The pattern does not militate against a_2-myeloma. (signed N. Harboe.

Asymmetry. Relativ viscosity increased, but not more than is consistent with the increase in total protein. Immuno-electrophoresis of serum. Obs. for a_2-myeloma with presence in the serum of ... paraprotein of the type β_2A-paraprotein (containing carbohydrate) which has a migration rate corresponding to the a_2 range. Inactivation of third part of complement, hypohaptoglobinemia, hypoalbuminemia. (signed J. Clausen)

Polysaccharide staining according to Harboe and Hansen (of serum after paper electrophoresis) a_1 6 ... a_2 59 ... β_1 24 ,, β_2 3 ,, γ 8 %.

Urine electrophoresis (fig. 3): as in normal urine. A protein excretion of 2.5 mg/hour is within the normal range. Immuno-electrophoresis of urinary protein: Abnormal γ-component (M-component with β-mobility. It seems to present a considerable number of antigenic groups, which can react with anti-γ. (signed J. Clausen)

Ultracentrifugation of serum showed the following distribution: = 12.4 7.3 (macro) = 6.3 22.8 (glob.) = 4.2 69.9 (alb.) Macro-components are markedly visible possibly in more than one size but the main component has = 12.4 and constitutes 7.3. The normal globulin peak is, perhaps, somewhat large though not unusually so. (signed R. Djurtoft)

Sternal marrow: 15 ... normoblastic erythrocyte precursors. "Within the myelopoesis the picture is dominated by plasma cells (33) varying in shape and size of which fairly large percentage are typical. (signed Emmerik Jensen)

X-ray examinations: Heart and lungs: no infiltrations in the lungs, both phrenicocostal sinuses normal, the heart slightly increased in breadth.

Ribs: No areas of decreased density.

Skull: Normal with no areas of decreased density.



Fig. 1 Serum electrophoresis. Negative side to the left, positive side to the right.

Laurell (5) describes one case, and has seen two more patients with α-myelomas (personal communication) All three cases belong immunologically to group β_2A.

Janssen (3) found no α_2-myelomas in 36 electrophoretic analyses. Harboe (2) likewise found no myelomas with α mobility among analyses from 63 patients. By subsequent examinations of sera from about 300 patients with myelomatosis the same author (personal communication) observed no α_2-myelomas either but in a single case a mobility within the range between α_2- and β globulin The results of the electrophoretic analyses left no doubt about the diagnosis of myelomatosis.

Thus, among the cases of α_2-myelomatosis described in the literature no more than six seem to be certain Two more have been heard of but are not published

Case report

Woman, aged 59 (Medical Out Patient Clinic) Previously in good health, apart from an attack of cholelithic pain 10 years ago Menopause at the age of 49 with no troubles. She had four normal deliveries from 35 to 17 years ago.

In Jan 1961 the patient consulted her own doctor owing to fatigue. After having been treated with iron tablets for 2 months she was referred to the Medical Out Patient Clinic owing to anemia and a high ESR. Apart from the fatigue and transitory dizziness she had no complaints. She had never had palpitations or precordial pain no pulmonary complaints. No dyspepsia, the bowel movements and urination had always been normal. There was no pain in muscles, and particularly no back pain.

Physical examination. State of nutrition above average. Slight pallor Apart from mild crural varices no abnormalities were found.

Other examinations Height 152.1 cm sitting height 81.8 cm. Weight (without a coat) 66 7 kg

Urine protein 0 glucose 0, nitrite-reaction 0.

Micr nothing abnormal. Hb 9 1 g%. Erythrocytes 2 98 mill./mm³ Color index 1 0? Leukocytes 2,840/mm³ Differential count (300 cells counts) 43 % neutrophils, 2 eosinophils, 4 monocytes, 46 % lymphocytes, 1 % myelocytes and 4 atypical cells.

ESR 101 mm in the first hour AST 40, ASH < 2 000.

Serum iron 57 μg% Ewald's test meal (30 min.) amount 35 + 17 ml, Congo/Phenolphthalein = 26/51 Feces No blood (× 3)

Total protein 8.6 g° (refractometric method)

Serum electrophoresis (Tiselius-electrophoresis) (fig. 1) albumin 40 1/2 % α 38 %

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Metabolic Studies in Clinical Magnesium Deficiency

By

Villy Posborg Petersen

This paper describes clinical and physiological findings in a patient with chronic magnesium deficiency which was part of a severe malabsorption syndrome. It also reports a series of studies which indicate that magnesium deficiency was associated with metabolic abnormalities affecting the renal and intestinal transport of several other electrolytes.

Case report

The patient was a 34-year-old man, who was admitted to this department for the first time on January 1, 1960 complaining of stiffness and painful paresthesias in both hands and feet, abdominal pain, diarrhea and weakness.

The patient had been in good health until 1948, when he began to suffer from episodic abdominal pain and diarrhea. He was admitted to another hospital in 1952, and from there was transferred to the surgical department of this hospital, where a laparotomy was performed. Regional enteritis was found in two segments of the small intestine. A proximal lesion originated at about 1.3 m from the duodeno-jejunal junction and had an extension of 1 m. The distal lesion covered about 70 cm of the terminal part of the ileum. Resection of the proximal lesion with end-to-end anastomosis was carried out while the distal lesion was left untouched. Microscopic examination of the excised intestine revealed severe inflammation involving all layers of the intestinal wall. The mucosa was almost completely replaced by a thick layer of fibrinous material and leukocytes. Massive infiltration with lymphocytes, plasma cells and eosinophils was present in the muscular layers and serosa. Scattered granulomas consisting of slender epithelioid cells and giant cells were also observed. A lymph node showed reticular hyperplasia, infiltration with plasma cells and eosinophils, and several small granulomas as in the intestinal wall.

No striking improvement followed the operation, and during the next six years he was admitted to hospital seven times with essentially the same complaints as before. During one of these stays he was critically ill with fistulas to the abdominal wall, and with ascites and edema due to thrombosis of the inferior vena cava. In 1958 a second laparotomy was performed. At the site of the previously made anastomosis there was stenosis of the intestine which was bypassed

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Fig 3 Urin electrophoresis. The graph corresponds to that seen in normal urine No abnormal fractions are found.

Pelvis and lumbar spine Mild diffuse halisteresis, but no bone destruction

The patient has been seen regularly for control during the past 8 months at the Medical Out Patient Clinic. Her condition has remained unchanged As the patient feels relatively well, being less tired, observation of her will be continued. The hemoglobin level has remained unchanged, and the urine is still Heller negative

Summary

Myelomatosis in which the abnormal protein migrates within the α range has been described in only few cases. Previously published cases are cited and discussed No more than six seem to be certain

A case of α_2 myelomatosis in a female aged 59 is presented. The investigations include examination of bone marrow of serum with Tiselius-electrophoresis, immuno-electrophoresis, salting-out and ultracentrifugation which confirmed the diagnosis.

Acknowledgements

This investigation was aided by a grant from Statens Almindelige Videnskabsfond.

The ultracentrifugation was kindly performed by R. Djurtoft, Dr techn., Research Laboratory of the Carlsberg Breweries and the immuno-electrophoretic analyses were carried out by J. Clausen, M D University Institute of Biochemistry Copenhagen.

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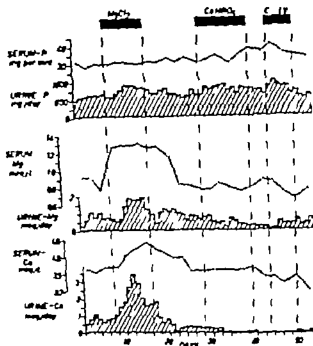


Fig. 1. Inorganic phosphorus, magnesium and calcium in serum and urine during 53-day study on constant dietary regime. Effects of supplements of oral magnesium chloride 63 mEq daily, oral calcium phosphate, 4 g daily and intravenous calcium gluconate 5 g daily in isotonic glucose.

serum magnesium ranged from 1.62 to 11 mEq/l with mean of 1.85 mEq/l. The 24-hour urinary excretion in most healthy subjects ranges from 5 to 15 mEq depending on magnesium intake. The titan yellow method could not be used for magnesium in food and feces. Their magnesium content was determined on ashed samples by flame spectrophotometry at 383 mμ, after precipitation of magnesium by ammonium phosphate according to a technique developed by Andersen and Jensen (1). Calcium was determined by complexometric titration using Eriochrome Black-T as an indicator (19, 23). The normal range for serum calcium by this method is 4.25 to 5.25 mEq/l. Inorganic phosphorus was measured by the method of Lundsteen and Vermehren (15). Nitrogen was determined by Kjeldahl analysis, and sodium and potassium by flame spectrophotometry. Analyses of food and feces samples for sodium, potassium, calcium, magnesium and phosphorus were done after wet ashing with concentrated nitric acid and 70 perchloric acid according to procedure developed by Hastrup (11).

Metabolic studies

Effects of administration of magnesium and calcium

The patient was placed on a constant diet which according to Danish food tables (9) contained 161 g each of protein and fat, 300 g carbohydrate, 2.0 g calcium and 3.5 g phosphorus. Calcium, magnesium and phosphorus in serum and urine are shown in fig. 1. Before any supplement was given, serum magnesium remained a low level of 0.7 to 0.9 mEq/l, and serum calcium at a level of 3.5 mEq/l. Oral administration of magnesium chloride as solution containing 63 mEq per day was followed by an immediate rise in serum magnesium to higher but still subnormal level of about 1.3 mEq/l, and an increase in urinary magnesium. Serum calcium rose to a low normal level, and renal excretion of calcium increased, reaching a peak of 5 mEq per day after five days magnesium treatment. Discontinuance of the magnesium supplement was followed by fall in magnesium and calcium in serum and urine to pre-treatment values.

The remaining part of this study, which extended over total of 53 days, comprised periods during which calcium phosphate was

by a side to-side anastomosis, but not resected whereby a blind loop was formed. About 50 cm of the grossly abnormal terminal ileum was removed and the remaining part anastomosed to the ascending colon Thus, the patient was left with an intestinal canal consisting of a normal duodenum a short length of jejunum including a side to-side anastomosis and a blind loop, followed by presumably about 2 m of intestine, of which the terminal 50 cm still was the site of chronic inflammation

After this operation his condition gradually improved The diarrhea subsided and his body weight increased by 20 kg However beginning in May 1959 a relapse had occurred and from then on diarrhea was constantly present On admission to this department on Jan. 1 1960 he had lost all the weight he had gained after the operation, and symptoms of tetany had developed.

Physical examination showed a thin and pale patient His body weight was 60 kg, his height 178 cm There were no signs of dehydration. The abdomen was moderately meteoristic and the subcutaneous veins on the lower part were slightly distended No masses were palpated Neither ascites nor edema could be demonstrated. Chvostek's and Trousseau's signs were present. Deep reflexes, gait and posture were normal He was mentally clear all the time and did not show convulsions or overt tetanic spasms.

The patient had two to four motions per day the stools were semifluid, pale and fatty and he passed up to 2 kg in a single day Fecal fat analyses showed a daily loss of 90 to 100 g fat of which 90 was hydrolyzed. Further evidence of malabsorption included a very low absorption of vitamin B_{12} as studied by the Schilling test. The urinary excretion of labelled B_{12} was 1.3 of an oral dose, and 0.8 when intrinsic factor was added (normal mean excretion 20 of the dose administered and lower normal limit 12) The serum content of vitamin B_{12} was 100 $\mu\mu$g/ml (lower normal limit 150 $\mu\mu$g/ml) A 5-hour xylose test showed an excretion of 2.4 g (normal 5—8 g)

Initially the serum calcium concentration was 2.25 mEq/l it subsequently ranged between 2 35 and 3.50 mEq/l before treatment. Urinary calcium was between zero and 1 mEq/24 hours. Serum magnesium ranged between 0.54 and 0.92 mEq/l, and the 24-hour excretion in the urine ranged between 0.5 and 1 0 mEq Serum inorganic phosphorus varied between 2.9 and 4 0 mg serum alkaline phosphatase was 7—8 K.A. units.

Hemoglobin was 10 0 g , red blood cell count $3.8 \times 10^6/\mu l$ hematocrit 33 MCV 87 μm^3 White blood cell count 8,600 μl, with a normal differential count. Bone marrow biopsy showed a normal normocytic erythropoiesis. Serum iron was 90 μg subsequently values down to 30 μg were found while the serum iron binding protein was within normal limits. Total serum protein was 5 7 g α -globulin 0.4, α_2- 0.6, β-0.9 and γ-globulin 1.9 g Total serum lipid concentration was 487 mg with a total cholesterol of 99 mg and total phospholipids of 118 mg Protein-bound iodine was 4 9 μg Blood urea 23 mg serum sodium 140 mEq/l, potassium 3.5 mEq/l, chloride 97 mEq/l and carbon dioxide 24 mEq/l.

The blood pressure was normal. The urine did not contain protein or sugar Liver and kidney function tests were within normal limits. Roentgenographic examination of the intestinal tract showed a rapid transit of the barium meal, which entered the large intestine after two hours. The contrast medium was irregularly scattered in markedly separated loops, and the mucosal folds appeared coarse and blunted. The terminal part of the ileum was short, stiff and uncoiled. Roentgenograms of the skeletal system revealed no bone lesions.

Methods

Determinations of magnesium in serum and urine were performed by the titan yellow method as described by Orange and Rhein (21) and modified by Andreasen (2) The same batch of dye was used throughout. The color readings were done in a Zeiss spectrophotometer at 540 μm. The presence of gluconate in serum interferes with color development yielding lower values for magnesium. Experiments with sera to which calcium gluconate had been added in tro showed that this effect was present only when the calcium content was raised by more than 1 mEq/L In twenty-six normal adult subjects

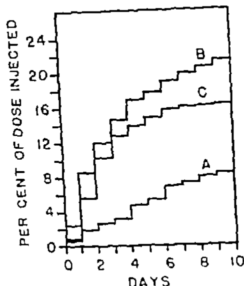


Fig 3. Cumulative excretion of fecal activity during 10-day period after intravenous administration of calcium chloride in control subject (A and in the patient off B and on C oral magnesium supplement

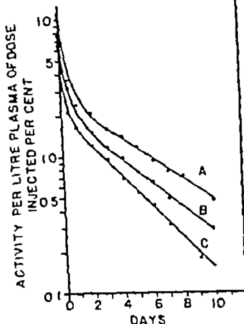


Fig 4. Plasma activities during 10-day period after intravenous administration of calcium chloride in the control subject A and in the patient off (B and on (C oral magnesium supplement.

excretion of magnesium does not increase after parenteral administration of magnesium (16) it did ppear to do so in this patient with grossly abnormal intestinal function, as shown by the following experiments

Intestinal loss of magnesium after parenteral administration

This experiment was carried out after the patient had been on magnesium therapy for several months. He was taken off magnesium supplement six days before the experiment as started and put on constant diet, which by analysis contained 23 mEq magnesium, 90 mEq calcium and 2.0 g phosphorus. The experiment included wo periods each of three days duration. Stools were collected and analyzed for each three-day period separately. During the second period he was given 32 mEq per day of magnesium sulfate intramuscularly. The results are given in table I which shows verage daily excretions during each period. It ppears that fecal magnesium increased by an average of 17 mEq per day corresponding to 50 of the quantity injected, while renal excretion rose to an average of 18 mEq per da. Serum magnesium increased from 1.37 mEq/l to a peak of 2.65 mEq/l, but fell rapidly to 1.56 mEq/l after discontinuance of parenteral injection of magnesium. The effect on urinary calcium excretion was again manifest by a large increase from 0.5 mEq in the first period to 7.9 mEq per day in the second. Serum calcium remained t fairly consta t level from 4.52 to 4.67 mEq/l throughout the experimen

I conclusion, no retention of magnesium occurred during this short-term administration of large doses of magnesium parenterally as both renal and intestinal excretions increased to such an extent that loses counter balanced the quantity injected.

Fate of radioactive calcium fter intravenous injection

The isotope studies to be reported below were performed with Ca⁴⁵ β- and γ-emitter with half-life of 4.7 days. Ca decay t

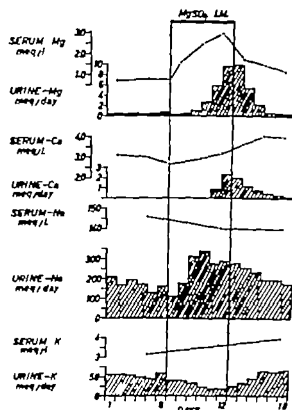


Fig 2 Magnesium, calcium sodium and potassium in serum and urine during an 18-day study on a constant dietary regime Effects of intramuscular administration of magnesium sulfat 16 mEq daily for 6 days.

given by mouth and calcium gluconate intravenously with intervening control periods. Supplements of tamins A and D were also given during these periods. Oral administration of calcium phosphate as CaHPO in daily doses of 4 g (466 mg Ca, and 360 mg P) was without effect on calcium and magnesium levels in serum and urine The calcium content in urine fell to zero and remained there for the rest of the period After an interval without mineral supplements calcium gluconate was given intra enously in daily doses of 5 g of the salt (445 mg Ca) infused over two hours in 1 litre of isotonic glucose (blood samples for analysis were taken before the infusion) No changes occurred in serum or urine calcium. Serum magnesium showed a slight, steady fall, and the urinary excretion of magnesium a small rise, as compared with pre-treatment values. Serum inorgan c phosphorus remained con stant throughout until calcium phosphate was

Table I Effect of parenteral magnesium administration on urinary and fecal excretion of magnesium

	Without Mg supplement mEq/day	MgSO I.M. 32 mEq/day mEq/day
Urinary Mg	1	18
Fecal Mg	34	31

given when a rise occurred, which levelled off during the ensuing administration of calcium gluconate.

In summary administration of magnesium chloride by mouth produced an increase in the contents of magnesium and of calcium in serum and urine. Calcium salts had no effect on serum or urine calcium, while intravenous administration of calcium gluconate caused a slight increase in renal magnesium excretion and a slight fall in serum magnesium concentration.

Effects of parenteral administration of magnesium

The patient was kept on the same dietary regime as used in the study described above. Magnesium sulfate in 20 solution was given intramuscularly in a dose of 16 mEq per day for six days. Changes in magnesium, calcium, sodium and potassium in serum and urine are recorded in fig 2. Serum and urine magnesium rose to high levels during this treatment and fell immediately afterwards. As during oral magnesium administration, parenteral administration was followed by a rise in serum calcium concentration, and renal calcium excretion increased from 0.5 to 2 mEq per day It also appears that magnesium sulfate administration produced a sodium diuresis and potassium retention.

The total quantity of magnesium injected during the six-day period was 96 mEq. Excess of magnesium excreted during 10 days, until excretion returned to pre-treatment values, amounted to 35 mEq (after subtraction of a base-line value of 0.45 mEq per day corresponding to the daily average excretion in urin during three days prior to magnesium injections) This means that 61 mEq (64 %) of magnesium was retained, providing that increased intestinal loss of magnesium had not occurred. Whereas in normal subjects fecal

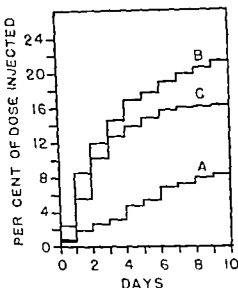


Fig. 3. Cumulative excretion of fecal activity during 10-day period after intravenous administration of calcium[47] chloride in control subject (A), and in the patient off (B) and on (C) oral magnesium supplement.

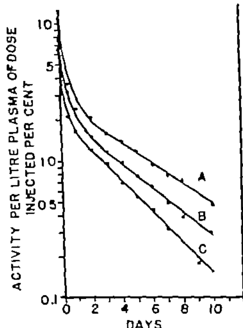


Fig. 4. Plasma activities during 10-day period after intravenous administration of calcium[47] chloride in the control subject (A) and in the patient off (B) and on (C) oral magnesium supplement.

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Intestinal loss of magnesium after parenteral administration

This experiment was carried out after the patient had been on magnesium therapy for several months. H was taken off magnesium supplement six days before the experiment was started and put on constant diet, which by analysis contained 23 mEq magnesium, 90 mEq calcium and 2.0 g phosphorus. The experiment included two periods each of three days duration. Stools were collected and analyzed for each three-day period separately. During the second period he was given 32 mEq per day of magnesium sulfate intramuscularly. The results are given in table I, which show average daily excretions during each period. It appears that fecal magnesium increased by an average of 17 mEq per day corresponding to 50 of the quantity injected, while renal excretion rose to an average of 18 mEq per day. Serum magnesium increased from 1.37 mEq/l to peak of 2.65 mEq/l, but fell rapidly to 1.56 mEq/l after discontinuance of parenteral injection of magnesium. The effect on urinary calcium excretion was again manifest by a large increase from 0.5 mEq in the first period to 7.9 mEq per day in the second. Serum calcium remained at fairly constant level from 4.32 to 4.67 mEq/l throughout the experiment.

In conclusion, no retention of magnesium occurred during this short-term administration of large doses of magnesium parenterally as both renal and intestinal excretions increased to such an extent that losses counter balanced the quantity injected.

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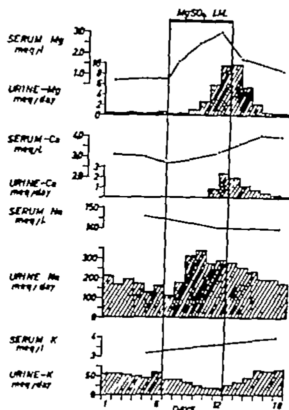


Fig 2 Magnesium, calcium, sodium and potassium in serum and urine during an 18-day study on a constant dietary regime Effects of intramuscular administration of magnesium sulfate, 16 mEq daily for 6 days.

given by mouth and calcium gluconate intravenously with intervening control periods. Supplements of vitamins A and D were also given during these periods. Oral administration of calcium phosphate as $CaHPO_4$ in daily doses of 4 g (466 mg Ca, and 360 mg P) was without effect on calcium and magnesium levels in serum and urine. The calcium content in urine fell to zero and remained there for the rest of the period. After an interval without mineral supplements calcium gluconate was given intravenously in daily doses of 5 g of the salt (445 mg Ca) infused over two hours in 1 litre of isotonic glucose (blood samples for analysis were taken before the infusion) No changes occurred in serum or urine calcium. Serum magnesium showed a slight, steady fall, and the urinary excretion of magnesium a small rise as compared with pre-treatment values. Serum inorganic phosphorus remained constant throughout until calcium phosphate was

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In summary administration of magnesium chloride by mouth produced an increase in the contents of magnesium and of calcium in serum and urine. Calcium salts had no effect on serum or urine calcium, while intravenous administration of calcium gluconate caused a slight increase in renal magnesium excretion and a slight fall in serum magnesium concentration.

Effects of parenteral administration of magnesium

The patient was kept on the same dietary regime as used in the study described above. Magnesium sulfate in 20 % solution was given intramuscularly in a dose of 16 mEq per day for six days. Changes in magnesium, calcium, sodium and potassium in serum and urine are recorded in fig 2. Serum and urine magnesium rose to high levels during this treatment and fell immediately afterwards. As during oral magnesium administration, parenteral administration was followed by a rise in serum calcium concentration, and renal calcium excretion increased from 0.5 to 2 mEq per day It also appears that magnesium sulfate administration produced a sodium diuresis and potassium retention.

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Table III Disposal of radioactive tracer after 10 days in control subject (A) and in patient off (B) and on (C) oral magnesium supplement

Experiment	Feces % dose	Urine % dose	Total % dose
A	8.57	26.74	35.31
B	21.50	18.17	39.67
C	16.35	13.57	29.92

in the patient, and that magnesium supplement did not reduce endogenous calcium loss.

Fate of orally ingested Ca^{47}

T experiments were performed in which radioactivity was measured in feces, urine and plasma after administration of the calcium isotope by mouth. One was done while the patient received no treatment until after the sixth da of the experimental period, when magnesium supplement was resumed (exp. B) I the second experiment (C the patient had been on magnesium therapy for several weeks, and continued on magnesium given as a suspension of magnesium hydroxid 112 mEq per day. Thirty μc of calcium^{47} chloride dissolved in tap water was given by mouth. The beaker was carefully flushed three times with water which was also swallowed. Afterwards the patient had his breakfast meal. During both experiments he was kept on constant diet containing 40 mEq Ca and 1,550 mg P. In exp. B serum magnesium ranged from 0.69 to 0.89 mEq/l, before magnesium was resumed, and afterwards rose to 1.59 mEq/l on the tenth da of the experiment. Urinary magnesium was 0.4—0.7 mEq per day and rose to 6—8 mEq after resumption of magnesium. Serum calcium aried between 3.47 and 4.00 mEq/l. Urine calcium was below 0.5 mEq per day and rose to 5—7 mEq after magnesium. During exp. C, serum magnesium was 1.40—1.50 mEq/l, and urinary magnesium 1.3—2.4 mEq per day. Serum calcium 3.83—4.08 mEq/l and urinary calcium 2—4 mEq per day.

As show in fig. 5, most of the activity left by the intestinal route. The cumulative excretion in the first five days was 97 % in exp. B and 86 in exp. C. Absorbed activity

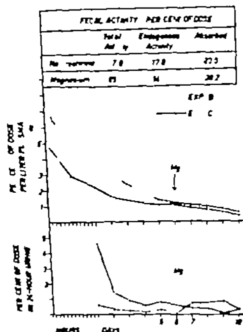


Fig. 5. F te of orally ingested calcium chloride in the patient off (B) and on (C) oral magnesium supplement. Uppermost, cumulative fecal excre tion in fiv days. Below plasma and urine ac tivities during 10-day period. In experimen C magnesium supplement was resumed af er the sixth day.

was obtained by subtracting from total activity the amount of tracer excreted through the intestinal wall as determined previously after intra enous administration of the isotope. The urinary excretion of activity was much higher in exp. C than in B during the first few days, whereafter daily excretion decreased slowly In contrast, only very small quantities ppeared in the urine, while the patient was off treatment. It should be noted that excretion in exp. B increased after resumption of magnesium supplement on the seventh day and actually rose to levels higher than those prevailing in exp. C. The higher urinary excretion in exp. C was associated with lower plasma ctivities; after resumption of magnesium therapy the plasma activity curves became parallel.

The difference in cumulative fecal excretion of tracer and calculated absorption, although not impressive might indicate that absorption

Table II. Intestinal loss of endogenous calcium in control subject (A) and in patient off (B) and on (C) oral magnesium supplement

Experiment	Fecal activity % dose in 5 days	Plasma activity % dose/l	Feces: plasma ratio	Plasma cleared of activity l/day	Endogenous Ca loss mg/day
A	5.14	1.14	4.5	0.9	89
B	10.49	0.80	13.1	2.6	205
C	9.26	0.60	15.4	3.1	248

Sc^{47} which is also radioactive and decays to stable Ti^{47} with a half-life of 3.4 days.

Two experiments were carried out in the patient, viz. one without magnesium supplement (exp. B) and one while he was given magnesium supplement 112 mEq per day by mouth as a suspension of magnesium hydroxide (exp. C). A parallel experiment was run in a control subject, a 60-year-old man admitted for angina pectoris, but free of symptoms during his stay in hospital. He presented no signs of intestinal or metabolic disease. Both subjects received the isotope from the same batch and at the same time. Twenty μc of calcium^{47} chloride in 5 ml of isotonic saline was injected intravenously. Radioactivity was measured in plasma, urine and feces in a well-type scintillation counter connected with a Tracerlab Superscaler. In both subjects stools were produced regularly and collected daily, homogenized and brought up to suitable volumes.

Intestinal loss of activity after intravenous administration is evident from fig. 3, which shows the cumulative fecal excretion of tracer over a 10-day period. It is obvious that the patient lost considerably higher quantities than the control subject. This difference is especially apparent during the first 3 days, in which the patient lost about five times as much of the tracer as the normal control. The normal figures (exp. A) agree with previous experiments carried out with a different calcium isotope Ca^{45} (5, 24). The fecal activities were lower while the patient received magnesium supplement (C) than when he was off treatment (B). It is doubtful however whether this indicates a true decrease in intestinal loss of the tracer since plasma activities (fig. 4) were lower in experiment C than in B. A rough estimate of the intestinal capacity for excretion of the tracer may be obtained from the relationship between fecal activity excreted over a certain period and the plasma activity. This ratio has been calculated for the five-day period extending from the 3rd to the 7th day using the average plasma activity in this period. The figures obtained (table II) which express litres of plasma "cleared" from activity by intestinal excretion are much higher in the patient than in the control subject, and perhaps higher when magnesium was given than when he was off treatment. A quantitative evaluation in terms of mg calcium removed from plasma by intestinal excretion is, however, subject to possible errors. The fecal activity is not collected pari passu with excretion into the intestinal lumen, which means that the timing of the collection period in relation to the chosen plasma activity value may not be quite identical in each of the three experiments. If it is assumed, however, that the tracer does reflect calcium transport quantitatively, it can be calculated from the figures in table II and the concentrations of stable calcium in serum that the control subject lost 89 mg calcium per day by intestinal secretion, while the patient lost 205 mg per day in experiment B, and 248 mg in experiment C.

The pattern of disposal of radioactive tracer appears from table III which demonstrates the difference in partition between intestinal and renal excretion. The patient lost more activity by the intestinal route than through the kidneys, while the control subject lost in the stools less than one-fourth of the dose administered.

A cautious interpretation of these experiments implies that the transport of radioactive calcium from the intravascular compartment to the intestinal lumen was abnormally high

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As shown in fig. 5, most of the activity left by the intestinal route. The cumulative excretion in the first five days was 87 % in exp. B and 66 in exp. C. Absorbed activity

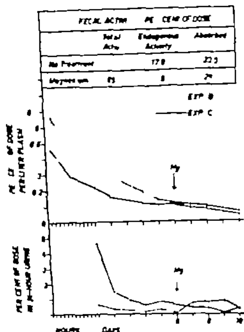


Fig. 5 Fate of orally ingested calcium⁴⁵ chloride in the patient off (B) and on (C) oral magnesium supplement. Uppermost, cumulativ fecal excretion in fiv days. Below plasma and urine activities during 10-day period. I experiment C magnesium supplemen was resumed after the sixth day

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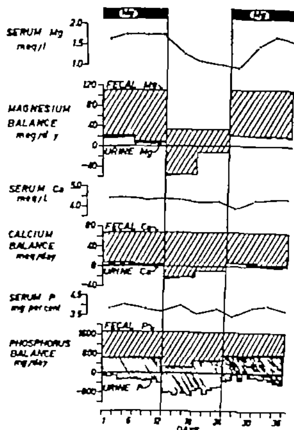


Fig 6 Metabolic balances and serum concentrations of magnesium calcium and phosphorus in the patient on and off magnesium supplement. Intake is plotted upwards from zero line and excretion, fecal and urinary down from the intake line. Positive balance is indicated by blank area above zero line and negative by columns below zero line

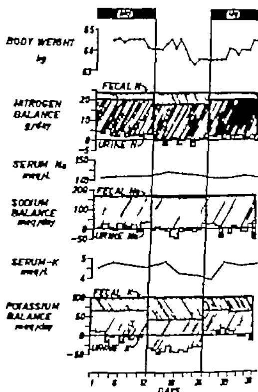


Fig 7 Metabolic balances and serum concentration of sodium and potassium, body weight and nitrogen balance in the patient on and off oral magnesium supplement. Data plotted as in fig. 6.

was actually improved during magnesium therapy and the higher urinary excretion might support this assumption More likely however the higher urinary levels of activity in exp. C were due to a greater filtered load of magnesium in the kidneys, as discussed below Such a supposition would also explain the rise in the excretion of tracer which occurred immediately after resumption of magnesium therapy

Metabolic balances

The patient received a constant, analyzed diet, containing 3 000 calories. Distilled water was used for drinking purposes. The balance experiment was preceded by a preliminary period of four days. The study included three experimental periods, each of 12 days duration. Feces was collected in 6-day periods, and carefully homogenized before samples were taken for analyses. Magnesium supplement was given as a suspension of magnesium hydroxide in periods 1 and 3, but withheld in the intervening period 2 By analysis the magnesium supplement contained 90 mEq per day The clinical condition remained essentially unchanged during the withdrawal period. He continued to have only one motion per day but the feces was more fluid and the average daily weight of fecal output increased from approx. 350 g to 550 g After resumption of magnesium stools became of the same weight and consistency as before

The balance charts in figs. 6 and 7 are so arranged that intake of dietary constituents and magnesium supplement is plotted upwards from the base line and excretion, fecal and urinary is then plotted down from the intake line.

It is seen that withdrawal of magnesium supplement produced a negative balance of magnesium and calcium, due to large intestinal loss of these ions. The urinary excretion of both substances was very small, as compared with the intestinal output, and although urinary excretion of calcium and magnesium fell to zero, this was without significance in terms of total balances. The fact that the intestinal output of magnesium and calcium was greater than the intake again indicates the occurrence of loss from endogenous sources. It also appears that intestinal losses decreased in the latter half of period 2, suggesting that some kind of adaptation to lower magnesium intake might take effect.

Phosphorus balance became negative, partly due to increased intestinal loss, but also as a result of increased urinary excretion of phosphorus. The average daily renal excretion of phosphorus was 888 mg in period 1, 1 191 mg in period 2, and 1,038 mg in period 3.

The fecal excretion of potassium increased from about 50 mEq per day to almost 60 mEq per day and potassium balance became negative after discontinuance of magnesium therapy. There was also a slightly increased urinary excretion of potassium. The average daily excretion of potassium in urine rose from 70 mEq in period 1 to 78 mEq in period 2, and decreased to 62 mEq per day after resumption of magnesium supplement.

Slightly negative balances occurred with respect to nitrogen and sodium, in both cases due to increased fecal excretion, while urinary output remained essentially unchanged. After withdrawal of magnesium, the daily fecal loss of sodium increased from 5 to 19 mEq, and fecal nitrogen excretion increased from 3.5 to 6.0 g per day. Resumption of magnesium therapy restored these and other balances to pre withdrawal levels.

Clinical course and follow-up

Before the patient was placed on magnesium therapy an experiment was carried out in which the effect of an acute injection of calcium was examined. Changes in serum magnesium and calcium were recorded after an intravenous injection of 40 ml of a 10 solution of calcium gluconate (336 mg Ca) given over a period of three minutes. The Trousseau sign was elicited by a sphygmomanometer cuff inflated to above systolic blood pressure and the time elapsed until carpal spasm developed was noted. It appears from fig 8 that the Trousseau sign gradually disappeared during the rise in serum calcium, while serum magnesium remained unchanged. Electrocardiograms taken before and at every 15 minutes for one hour after the injection were normal. The experiment demonstrates that tetany was relieved by a rising serum calcium concentration in the presence of a low serum magnesium level. On the other hand, the Trousseau sign remained positive while the patient was given a series of injections of magnesium sulfate, as long as serum calcium stayed below 3.5 mEq/l, and while serum magnesium rose to levels above normal

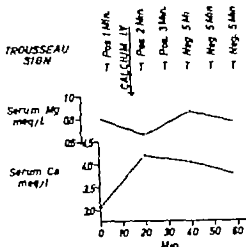


Fig. 8. Effect of an acute injection intravenously of calcium gluconate 4 g in 10 solution, on serum calcium, serum magnesium and the Trousseau sign. Result of test and time interval after application of cuff are recorded.

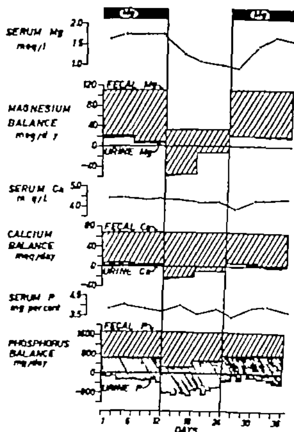


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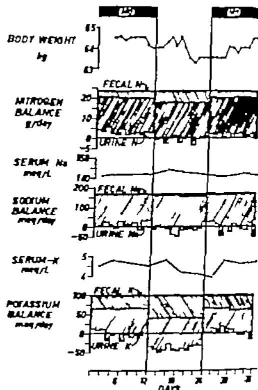


Fig 7 Metabolic balances and serum concentrations of sodium and potassium, body weight and nitrogen balance in the patient on and off oral magnesium supplement. Data plotted as in fig 6.

was actually improved during magnesium therapy and the higher urinary excretion might support this assumption. More likely however the higher urinary levels of activity in exp C were due to a greater filtered load of magnesium in the kidneys, as discussed below Such a supposition would also explain the rise in the excretion of tracer which occurred immediately after resumption of magnesium therapy

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The changes in calcium metabolism induced by magnesium therapy resulted in an increased serum calcium level and increased renal excretion of calcium. It might be assumed that increased serum calcium was the primary event, giving rise to a higher urinary excretion. While this may be true to some extent, it is obvious that an additional factor was responsible for the increased urinary calcium output. Mendel and Benedict (18) showed in animal experiments that parenteral administration of magnesium caused increased urinary calcium excretion. Several investigators (4, 27) have found the same phenomenon, without changes in serum calcium concentration, in normal human subjects, and also in cases of magnesium deficiency (8, 10). It is evident from these studies that the increased renal calcium excretion after magnesium administration is due to a renal tubular effect. Increased loads of magnesium will produce increased calcium excretion and vice versa (4). It has also been suggested that a similar relationship between magnesium and calcium may exist in the gut, which would mean that these elements were mutual inhibitors in intestinal absorption (10). For these reasons, it was important to decide from what source, bone or food, the increased calcium content in blood and urine originated. The results of the balance study indicate that magnesium supplement induced a decrease in net intestinal loss of calcium. If the secretion of endogenous calcium was unaffected by magnesium therapy as suggested by the fecal excretion after intravenous administration, it follows that calcium absorption was improved by this therapy. It is therefore concluded that calcium deficiency was counteracted by calcium from the diet, and not from skeletal stores. This conclusion is supported by the fact that bone lesions have not developed and that serum alkaline phosphatase has remained within normal limits after two years on magnesium therapy, on which urinary calcium excretion ranges from 100 to 200 mg calcium per day.

Potassium balance became negative following withdrawal of magnesium therapy due to increased excretion via the intestinal route as well as through the kidneys. The distribution of potassium between these excretory pathways was grossly abnormal, as intestinal excretion was almost as high as urinary excretion. Decreased absorption or increased secretion of potassium into the gut, or a combination of both, may be responsible for the increased fecal loss. Renal potassium excretion also increased when magnesium treatment was discontinued, and decreased after resumption. Parenteral magnesium administration also had the effect of increasing potassium retention by decreasing renal potassium excretion. However, this effect of high renal loads of magnesium on potassium excretion can be elicited whether magnesium deficiency is present or not (12, 27). It has been suggested that magnesium might enter into a tubular ion exchange mechanism, but experimental evidence in support of this hypothesis has not been forthcoming (14). The mechanism by which magnesium decreases renal potassium excretion remains unknown.

Withdrawal of magnesium supplement produced a slight increase in intestinal loss of sodium, while urinary excretion remained essentially unchanged. Parenteral injection of magnesium, however, was associated with a sodium diuresis. This latter feature is also part of a normal response to magnesium sulfate

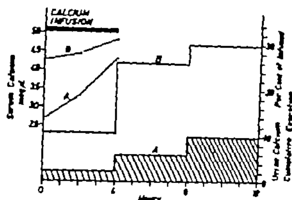


Fig 9 Results of calcium infusion test in the patient before treatment (A) and after several months therapy with magnesium supplement (B)

Continuous magnesium supplement administered as a suspension of magnesium hydroxide caused improvement of some of the signs and symptoms presented by the patient. The mild tetanic symptoms and abdominal pain disappeared The diarrhea subsided and stools became formed His body weight increased by 4 kg Serum calcium magnesium and inorganic phosphorus have been within normal limits on continuous magnesium therapy Several features, however of malabsorption has remained unchanged Firstly steatorrhea is of the same order of magnitude as before treatment The xylose test is abnormal as before The absorption of vitamin B_{12} iron and ascorbic acid remains unimproved by magnesium therapy as judged from the outcome of the Schilling test and serum analyses.

The condition of the skeletal system was evaluated by repeated radiographic examinations. Bone lesions have not developed and the serum alkaline phosphatase has constantly been within normal limits Results of two calcium infusion tests as described by Nordin and Fraser (20) are shown in fig 9 This test, which is thought to serve as an index of calcium deficiency is performed by intravenous infusion of a standard dose of calcium (15 mg/kg body weight) in isotonic saline over a four hour period and measuring the urinary calcium excretion in three consecutive 4-hour periods Experiment A was done before the patient was put on magnesium therapy and experiment B after he had been on continuous magnesium supplement for four months. As magnesium administration per se is able to increase urinary calcium excretion by an effect on the renal tubular reabsorption of calcium magnesium was withdrawn four days before experiment B was carried out. Urinary excretion of magnesium was below one mEq per day in both experiments. The 0—12 hour excretion in experiment A was less than 10 % of the dose infused while in experiment B 30 % was excreted in 12 hours. The normal range as given by the authors is 27—55 % excreted in 12 hours. All their patients with osteomalacia and one half of those with steatorrhea excreted less than 27 % of the infused dose. The results of the test suggest that the patient was in a state of calcium deficiency before treatment with magnesium supplement and that this deficiency was alleviated after treatment.

Discussion

Magnesium deficiency as defined by subnormal levels of magnesium in serum and urine which could be corrected by oral magnesium supplements was present in the patient herein described Low concentrations of magnesium in muscle have been reported recently in a case of idiopathic steatorrhea with hypomagnesemia (17) In the present case magnesium deficiency produced or aggravated metabolic abnormalities concerning several other electrolytes.

reported here was due to hypocalcemia and responded to intravenous calcium administration in the presence of a low serum magnesium. MacIntyre et al. (17) and Fletcher et al. (8) each reported one patient with malabsorption hypocalcemia and hypomagnesemia, in whom tetany also was relieved by calcium. So far magnesium deficiency has been observed in cases of malabsorption and in patients maintained for a prolonged time on magnesium-free parenteral fluid therapy. In both categories underlying disease and multiple metabolic abnormalities render it difficult to delineate clinical signs attributable to magnesium deficiency per se.

Summary

Studies are reported on patient with magnesium deficiency due to malabsorption, following extensive intestinal resection for regional enteritis. Several other metabolic abnormalities were present, including calcium deficiency and increased intestinal losses of potassium, sodium, phosphorus and nitrogen.

Administration of magnesium supplement corrected magnesium and calcium deficiencies, while calcium supplement was without any demonstrable metabolic effect except that intravenous calcium infusion increased renal magnesium loss. An attempt to elucidate the relationship between magnesium and calcium metabolism indicated that correction of the magnesium deficiency produced an increase in intestinal absorption of calcium, while an abnormally high calcium loss by intestinal secretion was probably unaffected. Increased intestinal losses of sodium and nitrogen, and increased renal and intestinal losses of potassium and phosphorus, were alleviated by correction of magnesium deficiency.

Acknowledgments

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administration (12 27) It is not clear whether this effect is due to the magnesium or to the sulfate ion as sodium excretion is unaffected by infusions of magnesium chloride or magnesium lactate (27) That some kind of relationship exists between renal transport of magnesium and sodium appears from the studies by *Hills* and his associates who showed that the rate of urinary excretion of magnesium and sodium changed in parallel when alterations in sodium excretion were induced by variations in salt intake and adrenocortical activity (13) Fitzgerald and Fourman (7) showed that experimental magnesium depletion in two human subjects was associated with a transient loss of potassium and a gain of sodium

Phosphorus balance became negative due to increased fecal and urinary excretion Intestinal loss of phosphorus ran parallel to that of calcium and magnesium possibly due to formation of slightly soluble phosphates of these metals. The increased renal excretion of phosphate may have been due to a parathyroid effect secondary to a decreasing serum calcium level. Urinary phosphorus rose from an average of 1 155 mg in the first half of the withdrawal period to 1,227 mg per day during the latter half both values being higher than in the preceding and following control periods.

Intestinal nitrogen excretion increased from about 3 to 6 g per day when magnesium was withdrawn Even though a faulty absorption may be responsible for the intestinal loss of nitrogen, it seems possible that an additional mechanism consisting in an abnormal escape from the intravascular compartment to the intestinal lumen may contribute to the total intestinal loss of nitrogen It has been demonstrated recently that intravascular albumin is lost in this way in cases of steatorrhea (22 26) In the present case, calcium and magnesium were probably lost into the intestine in this manner as suggested by abnormally high fecal excretions of these elements after parenteral administration, and by higher fecal excretion than could be accounted for by the dietary intake. In subjects without intestinal disease, fecal calcium excretion may temporarily exceed dietary intake, when the calcium intake is low (6) but with this exception no net loss of any dietary constituent normally occurs by intestinal excretion.

It is hardly possible to propose a unified concept which will account for all the metabolic abnormalities following magnesium deficiency in the present case The transit time of the intestinal content evidently increased as a result of magnesium therapy leaving a longer time for contact with the absorbing surface This factor does not, however explain the seemingly selective nature of the magnesium effect on net absorption. For the same reason it is difficult to imagine that intestinal losses were simply due to increased secretion of an inflammatory exudate or an increased flow of intestinal glandular secretions of a composition close to that of plasma or extra cellular fluid

Whether magnesium deficiency is associated with a characteristic clinical syndrome has been the subject of conflicting opinions Some workers have described a magnesium-deficiency tetany syndrome with low normal (25) or subnormal (3) serum calcium levels, which was relieved by magnesium but not by calcium intravenously Others (10) claim that tetany is not part of the magnesium deficiency syndrome. The mild tetany present in the patient

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From the Medical Department II (Head: Hans Hecksher M. D.) Municipal Hospital, Copenhagen, Denmark

The Influence of Tonic Neck Reflexes on the Activity of Some Muscles of the Trunk in Patients with Asthma and Emphysema

By

Erik Moltke and Arne P. Skouby

The present experiments form part of a study planned to elucidate the abnormal muscular behaviour in patients with asthma and emphysema.

During his studies on decerebrate rigidity Magnus incidentally disclosed that the distribution of the "tone" of the limb muscles in the preparation was dependent on the position of the head in relation to the trunk as well as to the gravitational field. This dependence on the position of the head was caused by impulses from proprioceptors in the neck and labyrinths (10). Later investigations on decerebrate animals showed that also the intercostal muscles, but not the diaphragm, were influenced by these reflexes (11).

Magnus-reflexes have been demonstrated in patients with brain damage (10, 15) and in normal infants (4, 14). They have not been established in intact adults, but in a few studies their presence was indicated under conditions with an increased activity of the motor units (5, 9, 16, 17). An increased permanent activity of some muscles on the neck and trunk, but not of the diaphragm, has been found in patients with chronic asthma and emphysema standing at ease (7, 8). It was therefore reasonable to assume that Magnus-reflexes would be demonstrable in such cases even when postural activity was reduced as far as possible.

This assumption was tested in the present investigation.

Method

The electromyographic measurements were performed as previously described (7). For all recordings an amplification corresponding to 10 μV = 1 mm and a paper speed of 5 cm/sec. were used.

All subjects were examined in the supine position on a firm couch. Recordings were obtained simultaneously from the diaphragm and from symmetrical sites over paired muscles of the trunk (infraspinati, latissimi dorsi,

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Table I. Influence of lateral flexion of the head in patients with asthma and emphysema

Diagnosis	No. sex	Infraspir.	Lat. dorsi	Serrati magni	Pect. maj.	Obliq. ext. abd.
Acute asthma	1 o	—	++	++	0+	0+
Acute asthma	2 o	0+	00	++	00	00
Acute asthma	3 ♀	0+	++	0+	00	00
Acute asthma	4 o	0+	0+	0+	00	00
Chron. asthma	1 o	00	++	++	00	00
Chron. asthma	2 o	++	++	0+	00	00
Chron. asthma	3 ♂	++	++	+0	0+	+0
Chron. asthma	4 o	0+	+0	0+	00	00
Emphysema	1 o	++	++	++	00	00
Emphysema	2 o	++	++	++	++	—
Emphysema	3 o	0+	++	++	00	00
Emphysema	4 o	+0	++	++	00	00
Emphysema	5 ♂	00	++	++	0+	00
Emphysema	6 o	0+	++	0+	++	00
Emphysema	7 o	++	++	++	00	00
Emphysema	8 ♂	+	++	00	++	+0

No (0) or definite (+) increase in electromyographic activity in ipsilateral surface muscles following lateral flexion of the head. For each muscle pair the response of the left sided muscle is given on the left in the column.

serrati magni, pectorales majores and obliqui externi abdominis) one pair at the time. The patient's head was firmly supported by the experimenter who also made sure that the subject relaxed as much as possible during the examination. Then he started a slow lateral flexion of the patient's head and maintained the new position for approximately half a minute. In this way changes in the position of the head in relation to the horizontal plane were avoided, thus excluding changes in labyrinthine stimuli. The electromyographic activity of the diaphragm and each muscle pair was recorded when the head was kept bent to the left, to the right and when kept in the normal middle position before and after each deviation. Thus each examination included at least five recordings from each of five muscle pairs and the diaphragm.

Control experiments were performed on two groups of medical students without cardiopulmonary disorders. The first group included five subjects without muscular or nervous complaints. The second group included three subjects with complaints from "muscular tension" and two nervous subjects. Both of them suffered from cool sweat and pallor in connection with the experiment.

In the *normal controls* no activity was recorded from the surface muscles whether the head was kept in the normal position or bent to the side. In the *tense or nervous controls* no or weak permanent activity was recorded from the superficial muscles when the head was kept in the normal position. In two of them lateral flexion of the head was followed by an increased activity in the ipsilateral latissimus dorsi. In one a decreased activity was obtained in the contralateral latissimus dorsi.

Results

Four patients with acute asthma, four with chronic asthma and eight cases with only emphysema were included in the study.

When the head was kept in the normal position no or a low grade permanent or respiratory activity was recorded from

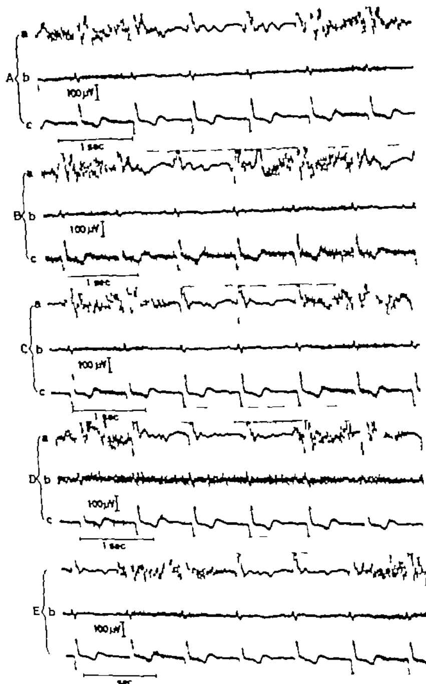


Fig. 1. Successive electromyographic recordings from the diaphragm (the right (b and the left (c) serratus magnus. Emphysema (table I, case 2
A, C, E: the head kept in the normal position. D the head is bent to the right.
B the head is bent to the left.

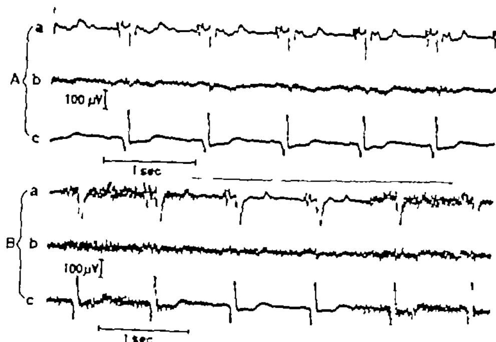


Fig. 2 Electromyograms from the diaphragm (a) the right (b) and the left (c) latissimi dorsi with the head kept bent to the right under voluntarily stopped respiration (A) and during usual respiration (B) Acute asthma (table I case 1)

the superficial muscles Lateral flexion of the head always caused or intensified electromyographic activity in two or several muscles of the ipsilateral side of the trunk. The muscles most frequently influenced were infraspinati latissimi dorsi and serrati magni This is seen from table I which also shows that the number of muscles involved in patients with only emphysema is at least as large as that involved in cases with acute or chronic asthma The increased activity in the ipsilateral muscles was often accompanied by a reduced activity in the contralateral muscles examined simultaneously

Fig 1 demonstrates the influence of lateral-flexion of the head on the activity of serrati magni in a case of emphysema In this case a definite increase of permanent activity was produced in the ipsilateral muscles. A release of permanent activity was observed whether the respiration was going on or stopped voluntarily This is demonstrated in fig 2, obtained from a case of acute asthma. Thus, the phenomenon was not dependent on respiratory activity controlled by will When the respiration was allowed to go on and the permanent activity produced by lateral flexion of the head was not too strong, an increase of inspiratory activity was often detected simultaneously in the recording from the ipsilateral areas

In the recordings from the contralateral areas the inspiratory activity was often reduced or even abolished This can be seen in fig 3 demonstrating the influence of lateral flexion of the head on the activity of pectorales majores in a case of emphysema. This effect on the respiratory activity could be demonstrated even if no permanent activity was present with the head in the normal position.

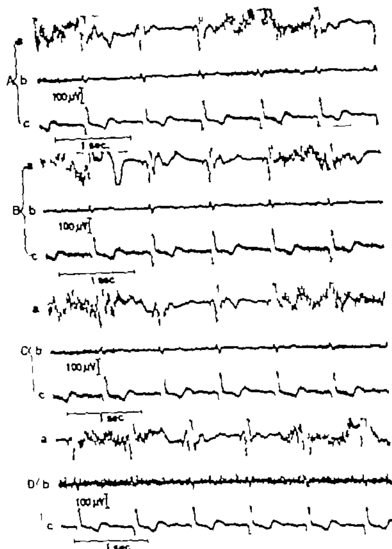


Fig. 3. Successive electromyographic recordings from the diaphragm ( ), the right (b) and the left ( ) pectoralis. Emphysema, table 1, case 2.
A, C: the head is kept in the normal position.
B: the head is bent to the left.
D: the head bent to the right.
Two later recordings of the series are given in fig. 4.

A released activity persisted for several seconds after moving the head to the normal position (fig. 4 A), while it was abolished instantaneously by lateral-flexion to the opposite side (fig. 4 B).

No influence on the electromyographic activity of the diaphragm was detected during the experiments.

Lateral-flexion of the head caused no visible movements of the trunk or

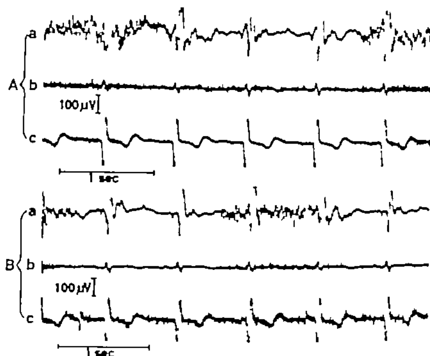


Fig 4 A recording with the head in the normal position for several seconds after bending to the right. B obtained just after bending the head to the left

shoulders and repeated procedures gave uniform results Thus, varying volitional activity was of no importance for the response.

Discussion

The experiments showed an abnormal permanent activity in the motor units of the surface muscles even in the resting patients This activity could be recorded directly in some cases. In others its presence was demonstrated by the influence of the tonic neck reflexes.

No definite conclusions can be drawn concerning the mechanisms underlying the abnormal activity of the motor units in the patients and the influence on this activity of the neck reflexes.

The permanent activity was not dependent on the respiratory activity influenced by will and a permanent activity was never demonstrated in the diaphragm Thus, it may be assumed that respiratory and permanent activities are initiated from different areas and integrated at the spinal level.

The distribution of permanent and respiratory activities in the motor units of the trunk muscles and the diaphragm of the patients examined was as in the decerebrate animal. In the latter the increased "tone" is assumed to be caused by an increased gamma motor activity via the spindle loop (6) As it is difficult to influence the phrenic motor neurones by afferent stimuli (2 3) an increased afferent activity caused by this or other mechanisms can result in an increased permanent activity only in the motor units of the superficial muscles.

An abnormal afferent impulse traffic might activate also the respiratory neurones of the reticular formation in the bulb (1 12 13) causing an intensified response to the respiratory stimuli. Due

to this and an increased excitability of the effector neurones at the spinal level, an inexpedient inspiratory and postural activity may easily become established.

Summary

When normal subjects were placed in the supine position on a firm couch and relaxed as far as possibl no electromyographic ctivity was recorded from infraspinati, latissimi dorsi, serrati magni, pectorales majores and obliqui externi abdominis whether the head was kept in the normal position or bent to one side.

I patients with asthma or emphysema examined under the same conditions little or no permanent or respiratory ac tivity was recorded from these muscles when the head was kept in the normal position Lateral-flexion of the head always produced changes in the electromyograms of two or several of the muscle pairs. These changes persisted as long as the new position of the head was maintained and disappeared several seconds after reversion to the middle position.

The changes included

1 *Permanent activity* which was elicited or intensified at the sid to which the head was bent and was abolished or reduced on the opposite side. This change was independent of espiratory activity controlled by will.

2. *Inspiratory activity* which was elicited or intensified t the side t which the head was bent and abolished or reduced on the opposit side. Such changes were recorded also without conspicuous per manent activity

In two of fiv tense" or nervous subjects without pulmonary disease similar changes were recorded thougl less pronounced.

The electromyographic activity of the diaphragm was never influenced by lateral-flexion of the head in controls or patients.

Acknowledgements

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W are grateful to the head of the Department f Physical Medicine Svend Clemmesen, M. D for working facilities and permission to use the DISA electromyograph.

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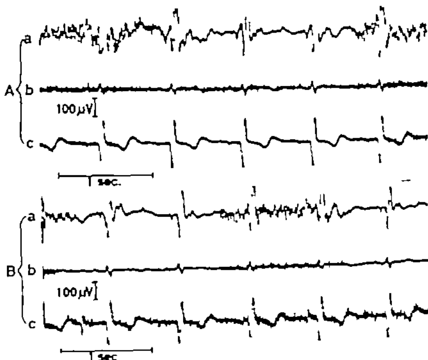


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From Medical department A, University Hospital (Head P. A. Owren, M. D.)
Rikshospitalet, Oslo, Norway

Studies on the Hemolytic Mechanism in March Hemoglobinuria

By

TORGEIR FLATMARK

Since its description by Fleischer (5) about 83 cases of this rare hemolytic disorder have been reported (20). The characteristic feature of this hemoglobinuria is its occurrence after physical exercise in the upright posture, particularly following walking and running. The hemolytic mechanism is still obscure. The purpose of this report is to present some observations in one patient, suggesting march hemoglobinuria to be a transitory acquired "erythropathia".

Case report

The patient was a 54-year-old man. For several years he suffered from duodenal ulcers, and in 1958 a subtotal gastric resection was performed. Since March 1960 slight Raynaud' phenomena in the fingers were observed.

1 February 1961 he observed one forenoon that the urine was darker than usual, resembling dark beer. Later the same phenomenon appeared 3—4 times following prolonged walking, and on one occasion the urine gave a strongly positive benzidine reaction and a positive test for protein. The patient was then referred to our department.

On admission the patient was underweight, but otherwise no pathological finding was demonstrated by the physical examination.

The urine was dark brown with a yellow-brown sediment after centrifugation. The supernatant gave a strongly positive benzidine reaction and a moderately positive protein reaction. The sediment gave macroscopically a positive Perls reaction with blue granules of hemosiderin demonstrable both extracellularly and intracellularly (in renal epithelial cells, granulocytes and granular casts). No erythrocytes were seen. The excretion of urobilinogen was normal. Electrophoresis of concentrated urine showed nearly the same fractions as in plasma (fig. 1). When the urine was saturated to 80 % with ammonium sulphate, the supernatant became almost colourless and gave a negative benzidine reaction. Like the electrophoresis this demonstrated the *presence of hemoglobin, and not myoglobin* (1).

The hemoglobin concentration was 12.2 g/100 ml and the red cell count 4.38 mill./mm³. MCHC was calculated to be 30 %. The reticulocyte count was 0.4 % and serum iron concentration 122 µg/100 ml. Bilirubin in serum was 0.6 mg/100 ml, and the direct and the indirect van den Bergh reaction were both negative. White blood cell count was 5,700/mm³ with a normal differential count,

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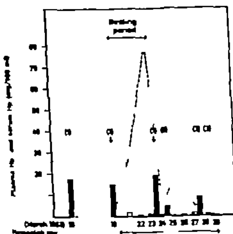


Fig. 2. Variations in the concentration of plasma-hemoglobin (columns) and serum-haptoglobin (dotted line) and in the excretion of hemoglobin in the urine. The open columns correspond to the values at 8 a.m. and the dark columns to the values following exercise. The arrows indicate exercise such as walking in town (1), load on tread-mill (2) and on ergometer cycle in horizontal position (3) (see text).

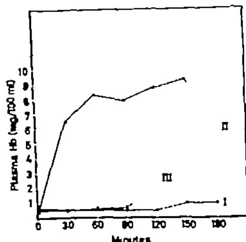


Fig. 3. The variations in the plasma-Hb with different degrees of exertion on three consecutive days. I. The patient walking on a tread-mill at a rate of 2 km/h. II. As in I, but the patient walking at a rate of 4 km/h. III. The patient on a cycle ergometer in horizontal position at a load of 100 kgm/min. The patient drank 100 ml of water at the start and every half an hour throughout the exertion period. Room temperature 23° C. The solid lines correspond to the exertion period and the broken lines to the period after exertion. No hemoglobinuria occurred.

and other peroxidase-active components were demonstrated by a freshly prepared benzidine-barium peroxide reagent (7) to which some sodium nitroprusside had been added to get a stable reaction product of benzidine blue.

Hemosiderin was stained by a freshly prepared solution made by mixing one part of 2 % potassium ferrocyanide with one part of 2 % hydrochloric acid.

The *osmotic fragility* was estimated in heparinized blood according to the method described by Papart et al. (16).

Acid hemolysis (Ham's) test. A 10 % suspension of washed erythrocytes was incubated at 0° and 37° C, respectively, for 1 hour in fresh normal compatible serum acidified to pH 6.5 with about one-tenth volume of 0.2 N HCl. The hemoglobin concentration of the supernatant was then read quantitatively.

The *heat resistance test* was performed according to the method described by Hegglin and Maier (10). 5 ml of blood was collected in a glass tube, incubated at 20° C and 24 hours later the concentration of hemoglobin in the supernatant was measured.

Autohemolysis was performed (A) in heparinized and defibrinated blood, respectively according to the method described by Selwyn and Dacie (9) and (B) in the following way. A 50 % suspension of washed erythrocytes was incubated at 37° C for 24 hours in the patient's plasma and in fresh normal compatible serum respectively. The tubes were then centrifuged and the hemoglobin concentration of the serum (plasma) was measured. Appropriate dilutions of the saved fresh serum (plasma) were used as blank solutions.

The *oxygen uptake of the erythrocytes* was estimated in presence of methylene blue according to the method described by Eldjarn and Bremer (3).

The *glucose consumption of the erythrocytes* was estimated in the following way. Washed erythrocytes were added to an isotonic phosphate Ringer buffer solution to yield a hematocrit of 50 %. Sterile glucose was added to



Fig 1 Migration pattern on paper electrophoresis of the protein fractions in the plasma and the urine Free hemoglobin and methemalbumin was demonstrated both in plasma and urine The Perls (Prussian blue) reaction showed the presence of hemosiderin in the urinary sediment No binding of hemoglobin to haptoglobin (Hp) could be demonstrated (ahaptoglobinemia) pH 8.6 (see text)

and platelets 255 000/mm A bone marrow aspiration revealed a low content of hemosiderin, but otherwise a normal morphological picture of the bone marrow cells. The concentrations of urea and creatinine in serum were normal, as was the electrophoresis of the serum proteins.

Following a long walk, a plasma Hb concentration of 17 1 mg/100 ml methemalbuminemia and ahaptoglobinemia were demonstrated (fig 1) Coombs reaction and the test for cold hemagglutinins were negative on three occasions. No hemoglobinuria could be induced by immersing the patient's feet in ice-water for 15 minutes. Serological tests for lues and specific tests for paroxysmal nocturnal hemoglobinuria were all negative.

During hospitalisation the tendency towards hemoglobinuria spontaneously disappeared, and it did not return until November 1961 when he again observed repeated, severe attacks of hemoglobinuria on walking The physical examination and the laboratory tests showed *principally* the same as at the first admission. During hospitalisation no hemoglobinuria was observed, but as soon as he left the hospital grossly visible hemoglobinuria occurred every day if he exerted himself. *However* the morning specimen was always clear and the urine remained clear if he rested at home.

Plasma was prepared from heparinized blood. Five ml of venous blood was collected in a test tube containing one drop of heparin (Heparin AL" 50 mg/ml) The blood was centrifuged (240 × g) for 5 min and the plasma pipetted off.

Serum from a healthy compatible donor was used. Venous blood was allowed to clot spontaneously in a glass tube at 37 C for one hour It was then centrifuged (240 × g) for 5 min and the serum pipetted off

Erythrocytes Heparinized venous blood (see preparation of plasma) was centrifuged five min (240 × g) and the plasma was discarded. The erythrocytes were washed three times in normal saline and the tube kept in ice-water until used.

The urine for electrophoresis was dialyzed against physiological saline and at the same time concentrated about 5 times by evaporation in a dialysis bag with a current of cold air from a hair dryer

The concentration of *hemoglobin in plasma and serum* was determined by near-ultra violet spectrophotometry according to the method of Harboe (9)

Hemosiderin was demonstrated in the urine according to Rous (18) and in the bone marrow according to Hansen and Weinfeld (8)

The concentration of *haptoglobin (Hp)* in serum was estimated by paper electrophoresis according to Nyman (15)

Paper electrophoresis. Separation of the protein fractions in plasma and urine was carried out in a LKB electrophoresis apparatus. A veronal buffer according to Michaelis (pH 8.6 and ionic concentration 0.125) was used. 10 mm³ plasma, 20 mm concentrated urine and 10 mm urinary sediment were applied, respectively The electrophoresis was run at room temperature for 14 hours at a current of 0 5 m Amp. for each 10 mm width of the filter paper (Whatman No 1)

After the separation the strips were dried and stained. The proteins were stained with Amido black 10 B (Merck") Hemoglobin

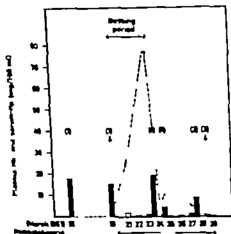


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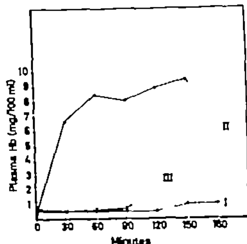


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Table I Autohemolysis of red cells measured according to Selwyn & Dacie (19) The patient was walking on a tread-mill at a rate of 4 km per hour for two hours on two consecutive days The concentrations of glucose and adenosine were 5.55 mM

	Lysis (in %) after incubation at + 37 C					
	24 hrs			48 hrs		
	Without any addition	With addition of glucose	With addition of adenosine	Without any addition	With addition of glucose	With addition of adenosine
At the start of the exercise	0.59 (0.15)	0.37 (0.09)	— (0.08)	1.66 (1.51)	0.63 (0.19)	— (0.40)
After exertion for 2 hrs	0.66 (0.26)	0.14 (0.14)	— (0.10)	1.11 (1.49)	0.47 (0.08)	— (0.41)
Normal range (Selwyn & Dacie (19))	0.05—0.5	0.0—0.4	—	0.4—4.5	0.03—0.4	—

Performed in heparinized blood.
Performed in defibrinated blood.

an initial concentration in the final volume of about 40 mg/100 ml. An aliquot was removed initially and at hourly intervals for three hours for determination of the glucose concentration by the method of Hultman (11) The glucose consumption was calculated as mM/1 000 ml/hour

Results

The degree of hemolysis in relation to physical exertion and artificial venous stasis

The patient rested *in bed* for some days The day after admission the hemoglobinuria had disappeared (fig 2) and only traces of protein were demonstrable During the following eleven days the urine was normal except for a continuous, but decreasing hemosiderinuria The concentration of haptoglobin (Hp) in serum increased from 0 to 80 mg/100 ml during a resting period of four days (fig 2) When the patient was allowed *out of bed* the Hp-value varied depending on his physical activity and the degree of hemolysis. The plasma Hb concentration was always < 1 mg/100 ml in the morning Following long walks it increased to a maximal value of 18.9 mg/100 ml but on no occasion did hemoglobinuria occur A moderate load on a *tread-mill* gave a significant pathological increase of the plasma Hb value (fig 3) However a greater load on a *cycle ergometer in horizontal position* gave no increasing of the plasma-Hb

During the second stay in hospital the plasma Hb increased from normal values to 40 0 and 55 4 mg/100 ml, respectively when the patient walked on a tread mill at a rate of 4 km/hour for 2 hours. This indicated a more active hemolytic mechanism than in March 1961 Proteinuria was observed but no hemoglobinuria. However the proteinuria was found only when *free* hemoglobin was demonstrable in the patient's plasma by paper electrophoresis. The test for orthostatic proteinuria was negative and during the exercise neither hypotension nor hypoglycemia was observed.

Table II. Autohemolysis of red cells measured by the increase in Hb concentration (mg/100 ml) in the supernatant after incubation at 37° C for 24 hours. The values obtained have been corrected to hematocrit of 50 %

	Erythrocytes incubated in	
	Patient's own plasma	Fresh normal, compatible serum
At the start of the exercise	148.7	74.0
After exertion for 2 hrs	73.5	125.4

Load, see table I

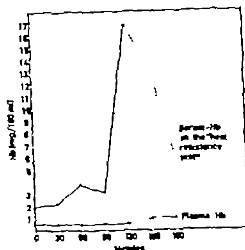


Fig. 4. *The increased hemolysis by incubation of* clotted blood at 20° C for 24 hours (the "heat-resistance test"). The patient was walking on tread-mill rate of 2 km/h. The solid lines correspond to the exertion period and the broken lines to the period after exertion.

In one experiment a *venous stasis* of 50 mm Hg was performed on the right arm when the patient worked on a cycle ergometer (100 kgm/min.) in horizontal position for one and a half hour. Venous blood was drawn from his left arm every half an hour and only a slight decrease of the plasma-Hb value was demonstrated.

Investigations in vitro on the hemolytic mechanism

As already mentioned, the serological reactions were all negative. A slight decreased osmotic fragility was demonstrated: the mean corpuscular fragility (MCF) was 0.40 % NaCl (before as well as following exertion) in agreement with a slight hypochromic anemia (MCHC 30). Autohemolysis tests were performed in heparinized and defibrinated blood respectively on two consecutive days following a moderate load on the tread-mill. From table I it is seen that the exercise produced no marked change in the lysis of the erythrocytes, this being always within the normal range. A slight decrease was observed in the 48-hour specimens, and in all incubations the autohemolysis was decreased by addition of glucose and adenosine to the same degree as in normal blood. However when the erythrocytes were incubated in normal compatible serum, the hemolysis was significantly *greater* in blood specimens taken after exertion than in those taken before (table II). Further when performing the "heat-resistance test" an increased hemolysis was demonstrated following exertion only at loads too small to give a pathological rise of the plasma Hb (fig. 4). Hemolysis did not occur either before or after the exercise in the acid hemolysis (Ham) test. The oxygen uptake in presence of methylene blue and the glucose consumption of the red cells were normal, and no difference was observed between the specimens taken before and after exertion.

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and no reticulocytosis, hyperbilirubinemia or urobilinuria developed. The hypochromic anemia present was due to a slight iron deficiency following the gastric resection and the intermittent loss of Hb and hemosiderin in the urine, as it disappeared following treatment with iron.

Summary

A typical case of march hemoglobinuria without hemolytic anemia is reported. The attacks of hemoglobinuria were always associated with proteinuria, methemalbuminuria and heavy hemosiderinuria, and free hemoglobin and methemalbumin were found in the plasma. During hospitalization a pathological plasma Hb value, but without hemoglobinuria, was repeatedly demonstrated even after slight exertions in the upright position. This lack of hemoglobinuria was shown to be due to an increased renal threshold of hemoglobin. This threshold depends primarily on the concentration of haptoglobin in plasma which in the patient increased rapidly during a resting period.

Investigations on the hemolytic mechanism indicate that it hardly can be a result of a local hemolysis confined to the vessels of the kidneys. An accelerated rate of the spontaneous hemolysis (autohemolysis) of the patient's erythrocytes as observed following exertion supports the theory of march hemoglobinuria being a transitory acquired erythropathia. The nature of this defect is still unknown, but it probably does not involve the carbohydrate metabolism of the erythrocytes. Further it is different from that in paroxysmal nocturnal hemoglobinuria (PNH) as the specific tests for this disease were all negative.

Acknowledgement

I am greatly indebted to Dr R. Nesbakken, Central Laboratory Rikshospitalet, for the determination of the oxygen uptake and the glucose consumption of the erythrocytes.

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Discussion

Our patient suffered from a paroxysmal hemoglobinuria and the attacks were produced only by physical exertion in the upright posture. No general systemic disturbance was demonstrated and the patient thus presented a typical case of march hemoglobinuria.

The attacks of hemoglobinuria were always associated with hemoglobinemia, but an elevated plasma Hb value without hemoglobinuria was repeatedly demonstrated even after slight exertions. When he rested in bed for four days the concentration of haptoglobin (Hp) in serum increased from 0 to 80 mg/100 ml and parallel to this also the "renal threshold" for hemoglobin. According to Boussier et al. (2) the minimum molecular weight of the Hb.Hp complex is 168 000 and such large protein complexes cannot pass through the filtration membranes of the glomeruli (13). Thus Hb cannot be eliminated via the kidneys until the concentration exceeds the hemoglobin binding capacity (i. e. the concentration of haptoglobin) of the plasma which in normal subjects varies from 30 to 190 mg/100 ml (15). However, when the concentration of Hb exceeds that of haptoglobin, free Hb will appear in the plasma. This Hb is filtered in part through the glomeruli but is not excreted in the urine until the amount of Hb filtered per unit of time exceeds the amount that can be reabsorbed by the tubules which in normal subjects varies from 0 to 54 mg/100 ml (12). Thus, the "renal threshold" for hemoglobin primarily depends upon the haptoglobin level of the plasma.

The mechanism of hemolysis in this disease is unknown. A relation to orthostatic proteinuria has been postulated but in our patient proteinuria occurred only when *free* hemoglobin was demonstrated electrophoretically in the plasma. Thus, the test for orthostatic proteinuria was negative. Further, the presence of hemoglobinemia, ahaptoglobinemia and methemalbuminemia without hemoglobinuria following prolonged walks, indicate that the hemoglobinuria, when occurring, can hardly be a result of a local hemolysis, confined to the vessels of the kidneys as first proposed by Robin (17).

An accelerated rate of the spontaneous hemolysis (autohemolysis) of the patient's erythrocytes following exertion could be readily demonstrated in serum from a normal, compatible donor but in the patient's own serum only at loads too small to give a pathological rise of the plasma Hb value. This suggests consumption of a serum factor (or serum factors) during in vivo hemolysis giving the serum a reduced hemolytic activity in vitro after exertion. This view is in accordance with the clinical observation that the rate of hemolysis during walking may diminish considerably after a certain point if the walk is sufficiently prolonged (6). The autohemolysis tests also demonstrate that the erythrocytes are more vulnerable following exertion than before and thus, march hemoglobinuria may be considered as a transitory acquired "erythropathia" as proposed by Martin and Kilian (14). The nature of this defect is, however, still obscure. It differs from paroxysmal nocturnal hemoglobinuria (PNH) since the specific tests for this disease were all negative. The strong inhibitory effect of glucose and adenosine on the autohemolysis and the normal glucose consumption of the erythrocytes suggest a normal glucose metabolism before as well as after exertion.

The extent of erythrocyte hemolysis in our patient was calculated to be negligible

and no reticulocytosis, hyperbilirubinemia or urobilinuria developed. The hypochromic anemia present was due to a slight iron deficiency following the gastric resection and the intermittent loss of Hb and hemosiderin in the urine as it disappeared following treatment with iron.

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Studies on the Secretin Test

By

Bent Collatz Christensen

The secretin test is a specific and sensitive test of pancreatic function. It depends on the use of a standardized reproducti e stimulation of the external pancreatic secretion and gives a quantitative estimate of the decreased pancreatic function.

Secretin was discovered n 1902 by Bayliss and Starling (4) who noted that an extract of dog jejunal mucosa with dilute aqueous hydrochloric acid injected intravenously produced stimulation of the external pancreatic secretion. Since then several biochemical groups have worked on preparing secretin and standardized secretin preparations. A historical and technical survey is given by Mutt (23) in his thesis 1959 The first non-toxic secretin preparations with a high biological acti ty that found wide spread clinical pplication (21) were produced by Hammersten Jorpes and Ågren (15) n the thirties. By the introduction of a new method for the preparation of secretin 1953 Jorpes and Mutt (16) succeeded in produci g secretin preparations of the hithert highest biological activity (17 18, 19) Recently the same authors have purified and analysed the secretin peptide (19)

The basic work with the secretin stimulation as a clinical test has been per formed by Lagerlöf et al (1 2 3, 21) Later Dreiling by using almost the same technique as Lagerlöf has carried out the most extensive investigations with the secretin test (8, 11 12, 13 14) and contributed towards a new way of diagnosing diseases of the biliary tract (9 10) A test of the pancreatic function with the use of both secretin and pancreozymin is described by Marks and Tompsett (22)

The effect of the secretin timulus is that the pancreas produces a secretion which has a high bicarbonate content and is relatively poor n enzymes. After secretin has been injected intravenously or intramuscularly the effect is recorded by analysis of the duodenal secretion which by means of a duodenal tube is aspirated quantitatively and without any mixing with gastric juice for a period of 60—80 min. The dosage of secretin is 1 clinical U/kg body weight which corresponds to a sub-maximal stimulation of the pancreas. The duodenal secretion

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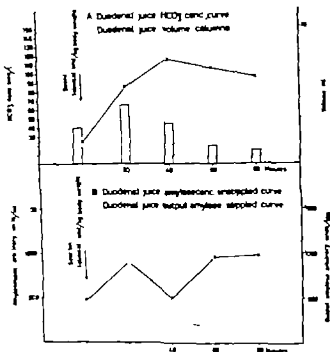


Fig. 1 A. Secretin response. Normal subject. The upper part of the diagram illustrates the changes in HCO_3 concentration (curve) and in the volume of the duodenal secretion (columns) after secretin is given intravenously. The lower part of the diagram shows the changes in amylase concentration and in amylase excretion.

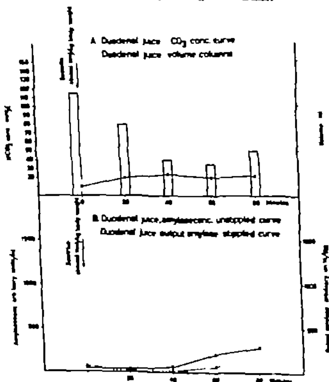


Fig. 1 B. Chronic pancreatitis steatorrhoea. The upper part of the diagram illustrates the low HCO_3 concentration (unstippled curve), the lower part of the diagram shows the low concentration and excretion of amylase in duodenal secretion (stippled curve).

is normally divided into fractions of 10—20 min and analysed for HCO_3 content volume per kg body weight and for enzymes. An isolated reduction of the bicarbonate concentration in the duodenal secretion for one of the 20 minute fractions to subnormal values, Dreiling's qualitative defect is a typical finding in chronic pancreatitis and is due to damage to the parenchyma of the pancreas (14). An isolated reduction of the volume of the duodenal secretion to subnormal values, Dreiling's quantitative defect is due to stasis of the main pancreatic ducts and is an important criterion in the early diagnosis of cancer of the head of the pancreas (14). Combined defect reduction of both the maximum HCO_3 concentration and of the volume to subnormal values, is observed by extensive damage to the parenchyma (14) after diffuse pancreatic inflammation as well as after prolonged obstruction of the pancreatic ducts.

A reduction of the total quantity of secreted amylase is often found simultaneously with a reduction of the maximum HCO_3 concentration in chronic pancreatitis. According to Dreiling (14) a reduction of the maximum HCO_3 concentration is found considerably more frequently in chronic pancreatitis (in 98 % of 135 patients) than a low amylase secretion (in 54 % of 135 patients) which is explained by the fact that secretin specifically stimulates the HCO_3 formation. With the combined use of secretin and pancreozymine Marks and Tompsett (22) found a reduced HCO_3 excretion as frequently as a reduced amylase secretion by a decreased pancreatic function.

The concentration of serum amylase before and after intravenous secretin injections, and the form of the curve of concentration is not considered of great value in the evaluation of the secretin response (13, 14, 22).

Methods

Two duodenal tubes are placed under X-ray control so that one of the tubes lies in the duodenum with the apex in the pars horizontalis whilst the other tube is placed in the stomach with the apex towards the pylorus. For the purpose of enzyme analysis the secretions are collected in suction flasks placed in iced water. Aspiration simultaneously from both tubes with a vacuum of 5—10 cm³ water pressure is carried out for one hour or longer until the amount secreted from the duodenum is under 20 cm³/20 min. Then 1 clinical U secretin/kg body weight, maximum 75 clinical U., are given intravenously. Preparation: Secretin Vitrum[1] (16, 17, 23). For the following 80 min. the aspiration is continued, and every 20 min. the collected secretion is isolated so that there are 4 fractions of duodenal secretion after the secretin injection each originating from 20 min. aspiration.

Chemical analyses

The quantities of duodenal and gastric secretions are measured and analysed for HCO_3, Cl, K, Na, pH and amylase. HCO_3 is determined by titration of the sample back to the original pH after expulsion of CO_2 by an excess of strong acid. All titrations are automatically performed by a titrator (type TTT radiometer Copenhagen). K and Na are determined by a direct reading Bechman flamephotometer. Cl is determined by potentiometric titration with silver nitrate.

Amylase content in serum and duodenal juice is measured by Street & Close's method (5, 24) slightly modified (20) where pure amylose is a substrate and where the process is observed by the bluish colour with iodine. In this work one amylase unit is determined as

$$\frac{\text{Extinction fall/m. under the given conditions}}{\text{The initial extinction}} \quad (20)$$

[1] Kindly supplied by A/S Alfred Benzon.

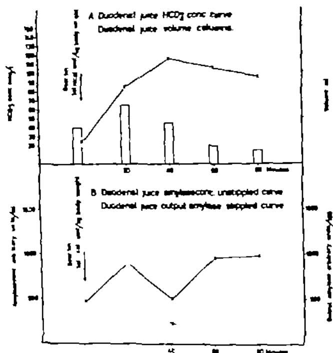


Fig. 1A. Secretin response. Normal subject. The upper part of the diagram illustrates the changes in HCO_3 concentration (curve) and in the volume of the duodenal secretion (columns) after secretin is given intravenously. The lower part of the diagram shows the changes in amylase concentration and in amylase excretion.

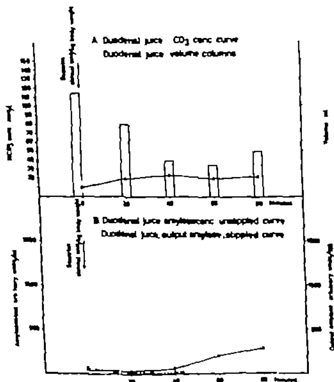


Fig. 1B. Chronic pancreatitis steatorrhoea. The upper part of the diagram illustrates the low HCO_3 concentration (unstippled curve), the lower part of the diagram shows the low concentration and excretion of amylase in duodenal secretion (stippled curve).

Table I Secretin test Group I Normal subjects (no of patients in brackets)

	Females	Males	Total
Duodenal juice max. conc. HCO (mEq/l)			
Mean values	88 (9)	116 (6)	100 (15)
S D	16.8	9.0	19.6
Range	62–115	101–127	62–127
Duodenal juice vol /kg/body weight (ml/kg)			
Mean values	2.8 (9)	2.9 (6)	2.9 (15)
S. D	0.9	0.9	0.9
Range	1.5–4 0	2.2–4.7	1.5–4.7
Duodenal juice total output HCO_3 (mEq)			
Mean values	10.63 (9)	20.40 (6)	14.54 (15)
S. D	5 0	7.6	7 7
Range	4.55–20.0	14.38–34.62	5.01–34.62
Duodenal juice total output amylase arbitrary (U/100)			
Mean values	2264 (7)	2160 (5)	2220 (12)
S D	1153	1257	1141
Range	993–4580	1311–2316	993–4580
Max. serumamylase conc. after secretin i.v arbitary (U/ml)			
Mean values	0.97 (6)	1.33 (6)	1 14 (11)
S D	0.36	0.50	0.45
Range	0.50–1.36	0.61–1.83	0.50–1.83

1 2 and 3 gives the significance as regards to 5 % 1 % and 0 1 % limit.

The enzyme concentration is expressed as U/ml. In order to avoid too large figures the total output of amylase is stated as the total secreted quantity of amylase divided by 100

The following points are taken into consideration

1 The maximum HCO concentration of the duodenal secretion is stated in mEq/l in one of the 20 min. fractions after injection of secretin.

2 The volume of the duodenal secretion per kg body weight indicated in ml/kg body weight.

3 The total quantity of HCO of the duodenal secretion indicated in mEq after injection of secretin (total output HCO)

4 The total quantity of amylase in the duodenal secretion after secretin injection is stated by the total quantity of units secreted divided by 100 (total output amylase)

5 The maximum serum amylase concentration after secretin injection is indicated by U /ml.

6. pH clarity colour and K concentration in gastric and duodenal secretions. These values help to decide whether the two secretions have been mixed. Duodenal juice is alkaline, clear and bile-coloured with a K concentration in the normal subjects of 5.0 in mean (observed range 2—9 mEq/l) Gastric juice is acid cloudy (mixed with saliva) and colourless with a K concentration in the normal subjects of 16 6 mEq/l in mean (observed range 10—20 mEq/l) Samples which indicate mixing of gastric and duodenal juice are rejected.

The secretin response is described in fig 1 1 A represents the findings by secretin test in a normal subject. The effect of the secretin given intravenously appears by an increase in HCO concentration volume and values of amylase secretion in the duodenal juice followed by a gradual fall in these values, slowest for the fall in HCO concentration. Amylase concentration values fluctuate in the first 40 min. rising and falling parallel with

Table II Secretin test. Group II group III and part of group IV (no of patients in brackets)

	Group II Chronic pancreatitis			Group III Stricture of the papilla of Vater	From group IV Dyskinesia
	Females	Males	Total	Females	Females
Duodenal juice max. conc. HCO_3 (mEq/l)					
Mean	72 (6)	71 (6)	72 (12)	83 (10)	92 (4)
S D	31	38	33	15.0	16.3
Range	24—144	8—117	8—117	62—103	68—104
Duodenal juice ml/kg body weight (ml/kg)					
Mean	3.5 (6)	2.7 (6)	3.1 (12)	3.3 (10)	3.2 (4)
S.D.	1.5	1.9	1.7	1.3	1.3
Range	1.3—4.8	1.1—5.7	1.1—5.7	1.5—5.8	2.1—4.8
Duodenal juice total output HCO_3 (mEq)					
Mean	12.55 (6)	12.71 (6)	12.63 (12)	12.38 (10)	12.48 (4)
S.D.	9.47	15.21	12.08	7.40	4.49
Range	2.24—23.63	0.60—39.67	0.60—39.67	4.12—26.99	6.44—16.39
Duodenal juice total output amylase arbitrary (U/100)					
Mean	376 (3)	2550 (3)	1463 (6)	2175 (5)	1139 (3)
S. D	300	2578	2003	871	155
Range	142—715	137—5266	137—5266	1141—3049	1041—1318
Max. serum amylase conc. after secretin arbitrary (U ml)					
Mean	1.22 (3)	1.83 (4)	1.57 (7)	2.69 (5)	1.29 (3)
S. D	0.05	0.43	0.45	2.58	0.18
Range	1.18—1.28	1.35 2.40	1.18—2.40	0.85—7.05	1.12—1.48

[illegible] and [illegible] refer to correspondent figures in table I

the volume and total output amylase In the last 40 min. the amylase concentration rises with falling volume and falling total output amylase

Material and results

The material involves 52 secretin tests carried out on 52 patients divided by diagnosis into the following groups:

I Normal subjects, 15 persons.

II Chronic pancreatitis, 12 patients.

III Stricture of the papilla of Vater 10 patients.

IV Mixed group, 15 patients suffering from various digestive disturbances.

Group I Normal subjects

Normal subjects consisted of 9 women whose average age was 22.8 years (19—37 years) and 6 men whose average age was 22.5 years (17—25 years). One of the patients was woman of 37 years who suffered from neu-

rosis cordis, whilst the other persons were healthy subjects, medical students and student nurses, who were paid for their assistance

As appears from table I there is a significant difference in the figures for men and women with regard to the maximum HCO_3 concentration in the duodenal secretion as well as in the total output of HCO whilst the other figures do not show any significant difference

Group II Chronic pancreatitis (table II)

The group includes 12 patients whose ages range from 19—72 years, with an average age of 51 years, 6 men and 6 women with an averag age of 50.2 and 51 9 years, respectively Eight of the patients had steatorrhoea, and 5 of the patients had previously been submitted to a stomach resection (Billroth I)

The diagnosis of pancreatitis had been operatively confirmed in 6 patients. In these as in the other patients the diagnosis had been made clinically before by a combination of few or several of the following criteria, steatorrhoea, pain, diastasuria and therapeutic effects of pancreatic enzymes.

The results appear from table II which shows that the various figures are of the same size for both sexes, except the figures for the secretion of amylase in the duodenal secretion The low figures for the women are due to the fact that the 3 observations derive from patients with steatorrhoea.

Compared with the results of the normal group it appears that the patients in the pancreatitis group had significantly lower values of maximum HCO concentration of the duodenal secretion most marked in men lower values of total output HCO in men (no significance) and significantly lower values of total output amylase in 4 patients with chronic pancreatitis with steatorrhoea (see below) The other figures show no definite difference from the figures for the normal group There is a tendency towards slightly higher values of the maximum serum amylase concentration.

Group III Stricture of the papilla of Vater (table II)

The group consists of 10 patients, all women whose ages range from 21—52 years with an average age of 35.8 years, 9 patients had previously been submitted to a cholecystectomy and all had severe postcholecystectomy pains, whilst 1 patient had not previously been submitted to cholecystectomy All 10 patients had suffered from severe hepatic colic, 6 had been jaundiced and 6 had had a rise of temperature whilst 3 patients had only had pain.

The diagnosis of stricture of the papilla of Vater was made during operation by probing the bile duct, and all 10 patients were submitted to the operation of transduodenal sphinchteroplasty The pancreas was normal in all 10 patients.

In comparison with the results of the women in the normal group the patients with stricture of the papilla of Vater showed no significant difference apart from the values of the maximum serum amylase concentration As this difference depends on one very high observation this difference is not significant.

Group IV Mixed group

The group consists of 15 patients. Of these patients 4 had postcholecystectomy pain, and one patient anomalia ducti Wirsungiani. Five patients with steatorrhoea will be discussed below whilst the other patients will not be discussed further

Four patients, who previously had been submitted to a cholecystectomy were considered to suffer from postcholecystectomy pains without recurrence of stone or stricture of the papilla of Vater owing to monosymptomatic hepatic colic of brief durat on and normal X ray and laboratory data.

As it appears from table II the results only differ a little from the results in the normal group apart from the low figures for the total output of amylase

Table III Secretin test (no. of patients in brackets)

	Chronic pancreatitis with steatorrhoea			P.G.S. with steatorrhoea	Idiopathic steatorrhoea		
	Females	Males	Total	Males	Females	Males	Total
Duodenal juice max. HCO conc. (mEq/l)							
Mean	66.5 (4)	59.3 (4)	62.9 (8)	94 (2)	108.5 (2)	112 (1)	110 (3)
S.D.	37.9	38.2	35.4	4.2	9.2	–	6.9
Range	24–114	8–99	8–114	91–97	102–115	–	102–115
Duodenal juice vol./kg/body weight (ml/kg)							
Mean	3.0 (4)	1.5 (4)	2.3 (8)	3.4 (2)	3.8 (2)	2.0 (1)	3.2 (3)
S.D	1.4	0.4	1.3	1.6	1.4	–	1.4
Range	2.1–4.6	1.1–2.0	1.1–4.6	2.3–4.5	2.8–4.8	–	2–4.8
Duodenal juice total output HCO_3 (mEq)							
Mean	9.68 (4)	3.65 (4)	6.66 (8)	14.0 (2)	16.04 (2)	12.36 (1)	14.81 (3)
S.D	10.8	2.3	7.9	2.6	1.9	–	2.5
Range	2.24–23.63	0.80–5.43	0.60–23.63	12.19–15.83	14.72–17.36	–	12.36–17.36
Duodenal juice total output amylase arbitrary (U/100)							
Mean	376 (3)	137 (1)	317 (4)	2169 (2)	2986 (1)	–	–
S.D	300	–	273	458	–	–	–
Range	142–715	–	137–715	1844–2493	–	–	–

1, 2 and refer to correspondent values in group I.

The patient with anomalia ducti Wirsungi- and at operation showed kink of the pancreatic duct near the ampulla with poststenotic dilatation, and caudapancreatico-jejunostomy by the method of Roux-Y was performed. Secretin test showed a normal HCO concentration (100 mEq/l) and a low volume (1.6 ml/kg body weight) by repeated tests. This finding corresponds to Dreiling's quantitative defect.

Of the 13 patients with steatorrhoea (table III), 8 patients suffered from chronic pancreatitis, 3 from idiopathic steatorrhoea and two from PGS symptoms. Of the pancreatic patients 5 had been submitted to a stomach resection (Billroth I); 3 patients had not earlier been operated on.

The diagnosis is confirmed by operation in 3 patients (only in one patient had a biopsy been performed). In these and in the other patients the diagnosis of pancreatitis is based on the occurrence of a few or several of the following characteristics: pain, steatorrhoea, diastasuria and the therapeutic effects of pancreatic enzymes. The diagnosis of idiopathic steatorrhoea is based upon some of the following characteristics: abnormal glucose-resorption, a sprue-like X-ray appearance of the small intestine, normal amylase quantities in the urine and serum, and therapeutic effect of gluten-free food. The diagnosis of PGS with steatorrhoea without pancreatitis is based on the finding of a normal pancreas at operation simultaneously with the occurrence of stea-

torrhoea, or on a finding of a normal pan creas at operation after performance of the secretin test.

Table III shows that in patients with chronic pancreatitis with steatorrhoea the figures for the maximum HCO_3 concen tration of the duodenal secretion the total output of HCO_3 and the total out put of amylase are significantly lower than the corresponding figures in normal subjects and lower than the corresponding figures in other groups of patients with steatorrhoea with the clearest significance of the low quantities of the total secretion of amylase.

It is with regard to the low values of total outpout of amylase that patients with pancreatogenic steatorrhoea most definitely differ from all other patients tested

Correlation between the total volume of the duodenal secretion, the total output HCO_3 and the max HCO_3 concentration

At the secretin test 3 things can be ex pected to be mutually dependent provided the technique of aspiration has been correctly carried out the maximum con centration of HCO of the duodenal secretion (max. HCO) the total volume and the total secretion of HCO (total output HCO_3)

The subjects are divided into 6 groups, first into normals, chronic pancreatitis and patients with other diagnoses, and each of these groups subdivided into m n and women. An analysis of variances show that it is admissible to summarize the results to the cor relation coefficients as stated below As will be seen all the correlations are signif icantly different from 0 The three values total volume max. HCO and total output HCO_3 have as a whole the same variation

The sam holds good for the variation of total volume and total output HCO_3, when max. HCO is kept constant and also the variations of the total output HCO and max. HCO_3 when the total volume is kept constant. Whereas the total volume and max. HCO_3 vary in the opposite direction, when the total output HCO_3 is kept constant.

Correlation coefficients	*Marginal*	*Conditional*
	$f = 36$	$f = 35$
Total volume — total output HCO_3	[3]0.91	[3]0.92
Total volume — max. HCO_3	[1]0.40	[1]–0.4[illegible]
Total output HCO_3 — max. HCO_3	[3]0.61	[3]0.66

f gives the number of degrees of freedom.
1 2 and 3 gives the significance as regards to 5 % 1 % and 0 1 % limit.

Fig 2 shows the proportion between the total volume of the duodenal secretion and the total output HCO_3. There is a significant dependence of 0 1 % between these, and the diagram shows that the HCO_3 secretion rises with rising volume. The same dependence is present when the correlation is calculated with a constant max. HCO_3.

Fig 3 shows the proportion between the total volume of the duodenal secretion and the max. HCO_3. There is only a significant dependence on a 5 % level between these two values, and the dia gram shows some indication that the maximum concentration of HCO_3 rises with increasing volume. The calculation of the correlation between the total vol ume and max. HCO_3, when the total output HCO_3 is kept constant, shows however that the max. HCO_3 decreases with increasing total volume

Fig 4 shows the proportion between total output HCO_3 and max. HCO_3 in the duodenal secretion. There is a signif icant dependence of 0 1 % level between these figures, and the diagram shows that the *total secretion of HCO_3 increases* with the increase of the max. HCO_3 concentration The same dependence is present, when the correlation is calculated with constant volume.

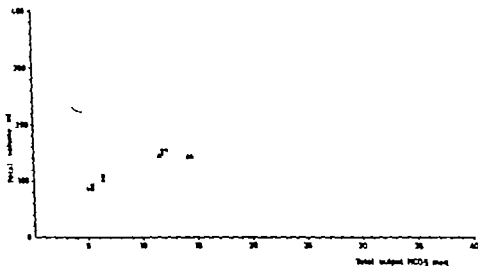


Fig. 2. Secretin test. The diagram presents correspondent, observed values of total volume and total output HCO_3 in duodenal secretion in 52 patients divided into chronic pancreatitis( ) normals(▲) and other patients ( ). Correlation coefficients see text.

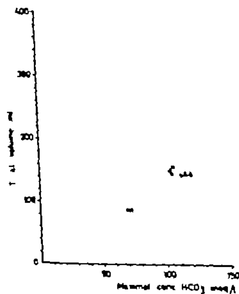


Fig. 3. Secretin test. The diagram presents correspondent, observed values of total olume and maximum HCO_3 concentration in the duodenal secretion in 52 patients divided into chronic pancreatitis( ) normals (▲) and other patients ( ). Correlation coefficients see text.

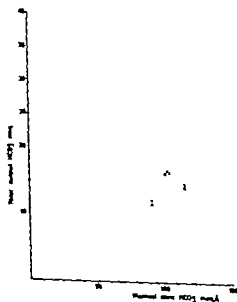


Fig. 4. Secretin test. The diagram presents correspondent, observed values of total output HCO_3 and maximum HCO_3 concentration in the duodenal secretion in 52 patients divided into chronic pancreatitis( ) normals (▲) and other patients ( ). Correlation coefficients are text.

Table IV Secretin test Normal values of max HCO_3 conc and vol /kg/body weight of duodenal juice Figures from different authors are compared with observations in the normal group of the present material

Author's secretin preparation	No. of normal controls	Duodenal juice max. HCO conc. (mEq/l)		Duodenal juice vol./kg/ body weight (ml/kg)	
		Different statistical data	Observed range	Different statistical data	Observed range
Lagerlöf (1)					
Purified secretin	13 1♀ 12♂	M 115 ± 3 σ = 11	94—137	M 3.8 ± 0.18 σ = 0.67	2.3—5.0
Diamond (6)					
Purified secretin	14	Not published	95—126	Not published	2.5—3.8
Dreiling (14)					
Crystalline	123	M 107 Calculated range 91—125	88—137	M 3.22 Calculated range 2.1—4.3	2.0—6.2
Secretin (Lilly)		Lower limit used 90		Lower limit used 2.0	
Own material					
Crystalline	15 9♀ 6♂	M 88 (9) sd 16.8 M116 (6) sd 9 0	62—115 101—127	M 2.8 (9) sd 0.9 M 2.9 (6) sd 0.9	1.5—4.0 2.2—4.7
Secretin (secretin Vitrum)	15♂+♀	M100(15) sd 19.6	62—127	M 2.9(15) sd 0.9	1.5—4.7

Discussion

1 Technique

Difficulties in performing the intubation correctly have been great, especially when working out the normal material on ambulant persons as 11 of 26 tests were not successful. There have not been any side effects from the secretion injection apart from a slight flush reaction in some of the cases. Nor were there any side effects after repeated injection of secretin Vitrum.

2 Secretin response

Normal response described under fig 1 A corresponds with the secretin response described by Lagerlöf, Diamond and Dreiling apart from the development of the concentration curve for amylase present in the duodenal secretion, which in the present material is parallel with the excretion curve for amylase for the first 40 minutes. The authors mentioned describe the amylase concentration curve as falling — then increasing, directly opposite to the volume curve. Secretin response in chronic pancreatitis with steatorrhoea corresponds to the findings of the authors quoted

3 Test results of the normal subjects

The results were surprising because of the significant difference in the response of the max. HCO_3 and the total output HCO_3 in men and women A similar observation is described in Lagerlöf's great publication (21) In a material of about 50 normal test subjects of both sexes a significant difference in the

output in men and women is shown with regard to the total volume of duodenal secretion and the total secretion of HCO_3 (stated as ml 1/10 n HCO) from a 60 minutes aspiration period. By adding some twenty patients more to the normal test subjects the difference between the two sexes was evened out. The number of normal subjects in the present material is small. As the period of aspiration after secretin injection and the calculation of the results are different (Lagerlöf calculates the reflux of the duodenal secretion when this occurs) a comparison with Lagerlöf's figures apart from the figures in the first publication (1) are also for these reasons not legitimate.

In table IV the results of the normal analyses from Lagerlöf (1) Diamond (6) and Dreiling (14) are stated, explaining the responses of max. HCO concentration in the duodenal secretion and volume per kg body weight at the same time as the applied secretin preparation is mentioned. Lagerlöf and Diamond have used purified, non-crystalline secretin (purified secretin) whilst crystalline secretin is used by Dreiling (preparation Lilly) and in these test subjects, also (preparation Vitrum) HCO is determined by Dreiling by the method of van Slyke, whilst the other authors use titration with 1/10 n NaOH after expulsion of CO with an excess of strong acid.

As far as the stated differences between the materials permit a comparison the responses for the max. HCO_3 concentration for men show in the present test group correspondent values with Lagerlöf's first material, which for the normal test subjects consisted of 12 men and 1 woman. The concentration of max. HCO_3 calculated as a total for men and women in the present test group is a little lower than Dreiling' values, and similar to those of Diamond, whose mean values are not stated. The outputs of volume per kg body weight in the present material are as a whole lower than in the materials quoted and the observed ranges lower

4 Chronic pancreatitis

The results of secretin test carried out in 12 patients with chronic pancreatitis is mentioned under the description of group II (table II) In two patients, 1 man and 1 woman, the secretin test gave a normal response with high values. Both patients had operatively verified pancreatitis (palpation without biopsy) and one patient had in addition steatorrhoea. As test errors cannot be traced, these false negative responses from two patients suffering from pancreatitis demand a certain reservation regarding the expectations, which can be held to the reliability of the secretin test, considering the applied technique.

5. Stricture of the papilla of Vater

Examination of 10 patients showed no depression of the pancreatic function. Analyses for bilirubin as indicated by Dreiling (10) have not been made.

6 Dyskinesia

Four patients with postcholecystectomy pains without suspicion of stone or stricture had a normal pancreatic function.

Steatorrhoea

The examination confirmed that the secretin test is suitable for the differential diagnosis of steatorrhoea as indicated by Lagerlöf, Dreiling et al., and recently emphasized by Marks and Tompsett's findings (22) The examination shows an indication that a low total secretion

of amylase directly reflects the digestive insufficiency A combined secretin pan creozymin test, which is indicated by Marks and Tompsett (22) will reveal probably more clearly than application of secretin stimulus alone the enzyme insufficiency in chronic pancreatitis with steatorrhoea.

8 The correlation between the total volume of the duodenal secretion, the total output HCO_3 and the max HCO_3 concentration

The 3 responses have shown the expected mutual dependence in the individual groups as well as in the complete material so that the total secretion of HCO_3 is bigger the bigger the volume of secretion and the max. HCO_3 concentration. The calculations showed that the bigger the secretion volume the lower the max. HCO_3 concentration

The demonstrated dependence between the volume HCO_3 secretion and HCO_3 concentration shows an indication that the aspiration technique with the use of 2 tubes instead of a double lumen tube has been uniform throughout the whole test series, so that almost the same percentage of the quantity secreted in each test has been aspirated. As tests with a reference substance have not been carried out it cannot be said whether the aspiration has been quantitative

Conclusion

The applied technique was the least distressing for the patient and gives comparable results from test to test. A significant difference in the maximum HCO_3 concentration and total output HCO_3 in the duodenal secretion is shown between men and women in the normal group The test can be used in the diagnosis of chronic pancreatitis and seems to give the most reliable information in men. By differential diagnosis between pancreatogenic and non-pancreatogenic steatorrhoea the secretin test is probably a reliable method as the total output of amylase is very low in pancreatogenic steatorrhoea.

The most important information in evaluation of the secretin test has been obtained by considering the values of maximum HCO_3 concentration, total output HCO_3 and total output amylase in the duodenal secretion.

Summary

The historical background for the secretin test and the most important criteria are briefly mentioned. A slightly modified Dreiling's technique with the use of 2 tubes is used Normal response, and response in chronic pancreatitis are presented. The results of 52 tests are stated The normal subjects (15 persons) showed significantly high values of the maximum HCO_3 concentration of the duodenal secretion and the total output HCO_3 in men In chronic pancreatitis (12 patients) one finds the greatest significance in lower values in men of the maximum HCO_3 concentration in the duodenal secretion and total output HCO_3 compared with the normal group. Ten patients with stricture of the papilla of Vater and 4 patients with post cholecystectomy pain showed a normal response. Thirteen patients with steatorrhoea are described separately In pancreatogenic steatorrhoea a low maximum HCO_3 concentration and total output of HCO_3 as well as a very low total output amylase was found. Corresponding values of the total volume of the duodenal secretion, total output HCO_3 and maximum concentration HCO_3 are analysed sta

tistically and show anticipated mutual dependence. Finally the results are discussed and are compared with results in other works.

Acknowledgements

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The laboratory tests are performed by the Department of Clinical Chemistry (Head P Astrup, M. D.) Rigshospitalet, Copenhagen, Denmark.

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Hypoglycemia Induced by Potassium Administration During Attacks of Periodic Paralysis

By

Uffe Sagild

Familial periodic paralysis, which was formerly believed to represent a clinical entity, has in recent years been shown to include at least three distinct genetically determined diseases: "classical" periodic paralysis, in which attacks of flaccid paralysis are associated with hypopotassemia, familial episodic adynamia (7, 10) and sodium-responsive normokalemic periodic paralysis (9).

In the first, and probably most common, of these diseases, a net potassium influx from the extracellular fluids into the muscle cells is believed to occur preceding and during the paralytic episodes (2, 3). This potassium shift may be induced by administration of glucose, and attacks may thus be precipitated experimentally.

An observation suggesting that, conversely, potassium administration during attacks may promote glucose entry in the cells is described in the following.

Material and methods

Two males with familial periodic paralysis, aged 33 and 35 years, both of whom have been described in a previous communication (10: cases VI, 5, 16 and VI, 5, 37) were studied (fig. 1). Four male hospitalized subjects in the same age group (36-45 years) with conditions unrelated to potassium or glucose metabolism were used as controls.

After an overnight fast the subjects emptied the bladder and were subsequently given 1 000 ml of a solution containing 50 mEq of potassium chloride and 100 mEq of sodium chloride in distilled water intravenously at a constant rate. The infusion was terminated within 60 to 70 min. Arterial and venous blood specimens (from the ear lobe and the antecubital vein) were obtained before the start of the infusion and again at 15 min. intervals during 90—105 min. Finally the subjects again emptied the bladder by spontaneous voiding.

The arterial blood samples were pipetted directly into freshly prepared zinc hydroxide and analysed in duplicate for glucose immediately following the test according to the Hagedorn-Norman Jensen method (8). All venous blood samples and the urine specimens obtained at the termination of the tests were analysed for potassium by flame photometry[1].

In each of the patients with familial periodic paralysis this test was carried out twice during spontaneous attacks which had begun during the previous night. These attacks were of

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[1] The analyses for potassium were carried out at the Central Laboratory (Head P. Astrup, M. D.), University Hospital, Copenhagen.

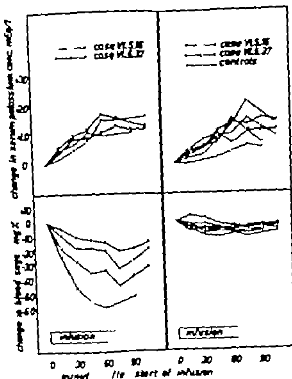


Fig 1 Changes in serum potassium concentration and blood sugar during and following intravenous potassium administration. Left Two patients with familial periodic paralysis during fou attacks. Right Same patients in interval and four control subjects.

moderate severity involving mainly the muscles of the back and extremities. In addition the test was carried out once in each of the patients in the attack free interval, and once in the control subjects. Furthermore, a control experiment during which isotonic sodium chloride was given rather than the mixture of sodium and potassium chloride was performed in patient VI 5 16 during a spontaneous attack.

Results

Potassium

The serum potassium concentration at the start of the infusion was low in the two patients with familial periodic paralysis during the four attacks (range 2 1—3 0 mEq/l) but rose to approximately normal values during the following hour. This was accompanied by a gradual return of normal muscular strength. In the same patients in the interval between attacks and in the four control subjects the initial serum potassium concentration was normal (range 3 6—4.3 mEq/l) and the increase in serum potassium concentration during the infusion was similar to that observed in the two patients during the attacks (fig 1)

Considerable differences were observed as regards the potassium content of the urine specimens voided at the termination of the tests. In the two patients during the four attacks the potassium content was low (range 1—11 mEq) while in the remaining six tests the range was 24—42 mEq of potassium.

In the control experiment during which isotonic saline was given to patients VI 5 16 in attack, the serum potassium concentration remained low (2.0—2.2 mEq/l) and the paralysis remained unchanged. The urinary excretion of potassium was 2 mEq during this test.

Glucose

In all tests the initial blood sugar concentration was normal (range 85—96 mg %) In the two patients with familial periodic paralysis during the four attacks the blood sugar decreased considerably during the infusion. The lowest blood sugar values were noted about 75 min. after the beginning of the tests. In one instance, the blood sugar was reduced by more than 50 per cent of the initial value. Coincident with the termination of the infusion of the blood sugar again began to rise (fig 1) No clinical symptoms or signs of hypoglycemia were observed.

In the same patients in the interval between attacks and in the four control

subjects a slight, but significant decrease in the glucose concentration of the arterial blood was noted. This was probably consistent with the expansion of the extracellular volume during the test.

A comparable slight decrease in blood sugar concentration was noted in the control experiment during which patient VI 5, 16 received isotonic saline.

Discussion

It is well known that a shift of potassium from the plasma and interstitial fluids into the cells may be induced by the administration of glucose alone or in combination with insulin. This has been used clinically in the treatment of hyperpotassemia (4) and experimentally in the precipitation of paralytic attacks in patients with familial periodic paralysis (1)

The details of the mechanism of this potassium shift are not well understood. According to older theories the cellular potassium uptake is linked to glucose uptake through the phosphorylation which initiates the metabolic breakdown of glucose or its conversion into glycogen. Intracellular accumulation of acidic phosphate esters of glucose then should cause a net influx of potassium to balance the increased intracellular acidity Recent works have thrown considerable doubt upon this concept. As shown by Zierler (11) insulin will increase the rate of potassium uptake in muscle even in a glucose-free medium, and in studies in man Andres et al. (3) have demonstrated that the intracellular influx of glucose and potassium from the extracellular space in response to insulin administration are dissociated temporally

It has been repeatedly shown that in familial periodic paralysis the fall in serum potassium concentration associated with the onset of attacks can be explained by a shift of potassium into the cells (2) and that potassium administered during attacks will be taken up rapidly by the cells during the recovery period (10) Thus the cells somehow display an increased avidity for potassium during attacks. In all experiments reported in this paper the rise in serum potassium concentration following potassium administration was of the same order of magnitude. However while the control subjects and the patients with periodic paralysis in the intervals between attacks excreted most of the potassium given rapidly in the urine, excretion was low in the two patients during the four attacks. A rough calculation based upon the assumption of an extracellular fluid volume corresponding to 20 per cent of the body suggests that a positive intracellular balance of the order of 20—30 mEq of potassium was built up at the time of termination of these four experiments, while in all the remaining this balance was only slightly positive or even negative.

In the same four experiments, and only in these, a mild but definite hypoglycemia developed during the same period Assuming a volume of distribution of glucose roughly equal to the extracellular fluid volume (6) the decrease in the "free" glucose pool was of the order of magnitude of 10—30 mM of glucose.

Although several mechanisms might be invoked to explain this phenomenon, among other things a decreased hepatic glycogenesis, it is tempting to speculate, that potassium entry in the muscle cells promoted a net influx of glucose in the same cells under the conditions of the experiment.

Summary

The blood glucose concentration in two patients with familial periodic paralysis and four control subjects was studied during and following intravenous infusions of potassium chloride in an aqueous solution made isotonic with sodium chloride.

The blood sugar remained approximately constant in the patients with periodic paralysis in the interval between attacks and in the control subjects.

During four spontaneous paralytic attacks, however the patients with familial periodic paralysis developed significant hypoglycemia during the potassium infusion.

This phenomenon is briefly discussed

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The Effect of Phenacetin Without Acetic-4-chloranilide on the Erythrocyte Lifetime in Phenacetin Habitués

By

Thorkild Friis and N. I. Nissen

It has been demonstrated in previous works that doses of 1.5 g per day of commercial phenacetin containing approximately 0.1 per cent acetic-4-chloranilide as impurity (4) given to phenacetin habitués *with* renal insufficiency provoked pronounced reduction in the erythrocyte lifetime as determined by the chromium technique. On the other hand phenacetin given to phenacetin habitués *without* renal insufficiency caused only a moderate or no reduction in erythrocyte lifetime (2) (The Pharmacopea Danica permits that phenacetin may contain up to 0.35 per cent acetic-4-chloranilide.) It was also found that *non-abusers* of phenacetin with renal insufficiency showed normal or slightly reduced erythrocyte lifetime, and that administration of phenacetin to such persons did not cause any further reduction (10) This would indicate a sensitizing mechanism, a postulate which is supported by the fact that prednisone counteracts the reduced erythrocyte lifetime (3) in patients with pyelonephritis who take phenacetin.

However Harvald et al. (5) have reported that phenacetin containing acetic 4-chloranilide increased the urine sediment of phenacetin habitués with reduced renal function as determined by Addis sedimentation count, while phenacetin without that substance did not exert the same influence It was therefore considered of interest to compare the effect of the commercial phenacetin product with that of phenacetin containing no acetic 4-chloranilide on the erythrocyte lifetime of phenacetin habitués without renal insufficiency

Material and methods

The material consisted of fifteen phenacetin habitués with renal insufficiency The designation phenacetin habitués is used for persons who have ingested at least 1.5 g phenacetin daily for a minimum of 1/2—1 year "Renal insufficiency" is used to describe conditions in which the serum creatinine value is > 1.4 mg/100 ml. In the majority of the patients, the erythrocyte lifetime was determined three times by means of the Cr^{51} technique described previously (3, 8 a.o.)

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Table I Data concerning 15 phenacetin habituals with renal insufficiency without administration of phenacetin containing no acetic-4-chloranilide (C)

No.	Sex	Age	Haemoglobin (> 80 %)			Sulphaemoglobin (≦ 2 %)			Reticulocytes (< 10 %)		
			A	B	Q	A	B	C	A	B	C
1	♀	40	68	—	49	0	—	1 4	—	—	7
2	♀	56	64	65	47	0	4.2	3.3	16	3	4
3	♂	58	67	70	67	2.8	1.8	2.9	5	25	18
4	♀	52	74	53	50	0	0.6	—	6	18	—
5	♀	53	72	45	64	0	6.6	0	4	15	11
6	♂	75	79	65	62	0	9.6	3.1	8	10	28
7	♀	60	83	80	90	0	0	0.5	4	17	8
8	♀	45	—	62	75	—	1 0	0	—	13	4
9	♀	52	52	42	73	3.5	9.0	6.5	10	50	20
10	♀	71	43	58	61	1 7	2.2	2.1	6	6	12
11	♂	67	79	56	76	0.3	1 0	0	8	10	18
12	♀	65	67	74	63	2.6	1.5	2.9	6	6	14
13	♀	64	—	70	57	—	0.7	2.1	—	8	6
14	♀	48	70	46	61	—	4.6	1.9	4	18	6
15	♀	60	96	57	106	0.3	8.7	0.7	6	5	3
Mean			70.3 ± 13.3	60.2 ± 11.3	66.9 ± 15.9	0.93 ± 1.33	3.68 ± 3.45	1.97 ± 1 78	6.9 ± 3.4	14.6 ± 12.0	11.4 ± 7.4

Blood transfusion given before determination of erythrocyt lifetime fresh type 0 erythrocytes from nor

with an interval of at least two months between each determination (the test takes three weeks to perform) No phenacetin was given between the test periods. The erythrocyte lifetimes were determined when no phenacetin was given (group A) when the ordinary commercial product was administered (group B) and when phenacetin containing no acetic-4-chloranilide[1] was used (group C) No blood transfusions were performed during the determinations. A few patients had been given transfusions before tests, and therefore healthy type 0 red cells were used in the determinations. This is permissible since t is the state of the plasma which is decisive in this connection for measuring the erythrocyte lifetime (3)

In addition to determination of the erythrocyte lifetime, repeated tests were made in order to measure th haemoglobin, haptoglobin (6 7 11) and creatinine concentrations in the serum, as well as the sulphaemoglobin concentration (1) and the number of reticulocytes using the techniques generally employed.

[1] Kindly provided by Messrs. Dumex, Copenhagen.

Results

The results of the examinations are shown in figs 1—7 and table I Neither the haemoglobin percentages nor the serum creatinine concentrations varied from each other in groups A, B and C.

However the sulphaemoglobin concentration values were different in the three groups. The figure was significantly higher statistically when ordinary phenacetin was given ($t = 2.58$ $0.02 > p > 0.01$) than when no drug was administered On the other hand there was no difference between groups B and C or

nacetin (A) during administration of ordinary phenacetin (B) and during administration of phenacetin

Haptoglobin (35—170 mg/100 ml)			Serum creatinine (< 1 4 mg/100 ml)			Erythrocyte lifetime $\frac{T}{2}$ days (24—36 days)		
A	B	C	A	B	C	A	B	C
412	—	0	7.52	—	7 70	25	—	5
69	32	35	2.48	2.31	2.33	27	18	7
434	220	300	6.40	8.65	8.40	31	10	10
217	174	—	7.65	5.45	8.50	25	17	16
147	—	120	6.31	6.72	7 45	38	18	16
89	—	293	8.35	7.57	11.90	23	16	16
228	370	232	1 71	2.21	1.74	35	16	17
—	7	153	—	3.31	2.54	—	19	19
113	10	—	9 83	7 73	7.24	44	11	20
349	37	243	8.73	6.40	7 78	26	11	20
51	0	8	10 80	13.02	13.10	30	11	21
—	408	292	1.25	2.19	3.27	35	24	23
—	—	398	—	7.50	4.40	—	14	23
—	19	0	5.30	6.60	3.06	30	17	28
—	370	242	2.84	2.21	2.04	40	17	30
232.7	149 7	178 8	6 03	5.65	6.21	31.2 ± 6 6	15.6 ± 3.9	18.0 ± 6.7

mal persons therefore used for determinations.

between groups A and C (t = 1.64 p > 0 1 and t = 1 64 p > 0 1) though the concentrations tended to be slightly higher in group B than in group C (table I)

As regards the reticulocytes, the number was greater in the habitués who were given phenacetin (B and C) than in those who did not take any phenacetin (A) The difference between groups A and B was significant (t = 2.14 0.05 > p > 0.02) while the difference between groups B and C was just under the level of significance (t = 1 82 0.1 > p > 0.05) There was no difference between groups B and C (t = 0.85 p > 0.1) as was also the case with the haptoglobin concentrations (table I)

As regards the erythrocyte half life-times (normal 24—36 days) groups B and C were significantly different from group A (t = 7 42 p < 0.001 and t = 5.83 p < 0.001) In group A only two were slightly reduced in group B all and in group C all except two were reduced (table I and fig 1) There was no definite difference between groups B and C (t = 1 13 p > 0 1) Fig. 1 also shows the values in three groups of patients examined in a previous study (10) viz, 16 phenacetin habitués without renal insufficiency who were given the commercial phenacetin product (D) 12 patients with renal insufficiency who were not phenacetin habitués but who were given phenacetin during the erythrocyte lifetime determination (E) and 11 patients of the same category who did not take phenacetin during the determination (F) The erythrocyte lifetimes for

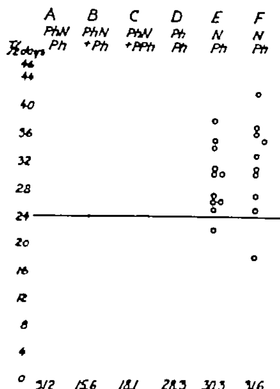


Fig 1 Biological half-lifetime of erythrocytes.

A Phenacetin habitués *with* renal insufficiency (PhN) *without* ingestion of phenacetin (— Ph)

B Phenacetin habitués *with* renal insufficiency (PhN) *with* ingestion of phenacetin (+ Ph)

C Phenacetin habitués *with* renal insufficiency (PhN) *with* ingestion of phenacetin containing no acetic-4-chloranilide (PPh)

D Phenacetin habitués *without* renal insufficiency (Ph) *with* ingestion of phenacetin (+ Ph)

E Non-abusers of phenacetin with renal insufficiency (N) *with* ingestion of phenacetin (+ Ph)

F: Same patients *without* ingestion of phenacetin.

Ordinate: Biological half-lifetime of erythrocytes in days.

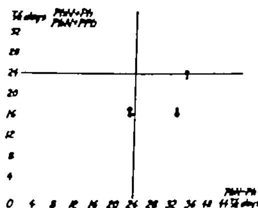


Fig 2 Relationship between erythrocyte biological half-lifetime with and without ingestion of phenacetin in phenacetin habitués with renal insufficiency

Abscissa Biological half-lifetime in days *without* ingestion of phenacetin.

Ordinat Biological half-lifetime in days *during* ingestion of phenacetin.

○ PhN + Ph Phenacetin habitués with renal insufficiency during ingestion of pheneacetin.

● PhN + PPh Same patients during ingestion of phenacetin containing no acetic-4-chloranilide

these patients are on the same level as those for group A. *It will thus be seen that both phenacetin with and without impurity caused by acetic-4-chloranilide reduced considerably the erythrocyte lifetime of phenacetin habitués with renal insufficiency*

Fig 2 shows the relationship between the erythrocyte half lifetimes when the "phenacetin nephropathic patients ingested phenacetin and when they did not. The reduction in erythrocyte lifetime when the patients took phenacetin bore no relationship to the values without phenacetin.

Fig 3 shows the relationship between the half lifetimes when the patients ingested the phenacetin without acetic 4-chloranilide and when the ordinary phenacetin was taken. In seven patients there was no difference One showed a pronounced reduction when the purified product was ingested by only a moderate reduction with ordinary phenacetin. The opposite was the case in six patients, two of whom even showed no reduction in erythrocyte lifetime when the phenacetin product without acetic-4-chloranilide was taken.

Fig 4 shows the relationship between the serum creatinine concentration and the half lifetime during phenacetin administration in groups B and C. The

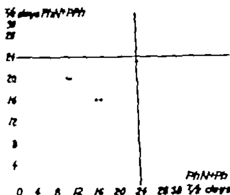


Fig. 3. Relationship between biological half-life-time during ingestion of phenacetin and during ingestion of phenacetin containing no acetic-4-chloranilide in phenacetin habitués with renal insufficiency

Abscissa: Biological half-lifetime in days during ingestion of phenacetin.

Ordinate: Biological half-lifetime in days during ingestion of phenacetin containing no acetic 4-chloranilide.

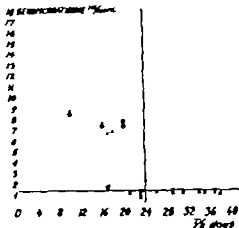


Fig. 4. Relationship between erythrocyte biological half-lifetime and serum creatinine during ingestion of phenacetin.

Abscissa: Biological half-lifetime in days.

Ordinate: Serum creatinine in mg/100 ml.

● PhN + Ph: Phenacetin habitués with renal insufficiency during ingestion of phenacetin (B).

○ PhN + PPh: Same patients during ingestion of phenacetin containing no acetic-4-chloranilide (C).

× Ph + Ph: Phenacetin habitués without renal insufficiency during ingestion of phenacetin (D).

values for the phenacetin habitués without renal insufficiency given ordinary phenacetin are also shown in the figure (10). It will be seen that increasing serum creatinine is accompanied by decreasing $\frac{T}{2}$ in the case of the ordinary phenacetin, as mentioned previously (2), but that there was a considerably larger deviation as regards the phenacetin without acetic 4-chloranilide.

Fig. 5 shows the relationship between the sulphaemoglobin concentration and the half-lifetime. When there was decreasing $\frac{T}{2}$ there was a tendency to increased amounts of sulphaemoglobin, particulary as regards the phenacetin with acetic-4-chloranilide. The values for the phenacetin habitués without renal insufficiency mentioned in the previous work (10) are also included in the figure. These are on the same level as those for the patients with renal insufficiency, and thus this aspect is of little importance as regards the sulphaemoglobin concentration.

Fig. 6 and 7 show the corresponding relationships between the reticulocyte count and haptoglobin concentration and the half-lifetimes. As regards the reticulocytes, it was found that a reduction in half-lifetime was connected with increased number of reticulocytes. With regard to the haptoglobin, it was seen that all those (except one) who had reduced values had also reduced erythrocyte lifetimes, but that many with reduced erythrocyte lifetimes had normal and even increased haptoglobin values. This may be due to the presence of kidney infection (7, 11).

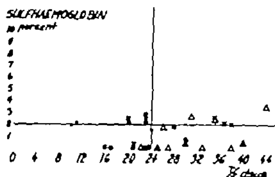


Fig 5 Relationship between erythrocyte biological half-lifetime and sulphaemoglobin concentration in phenacetin habitués with renal insufficiency

Abscissa Biological half-lifetime in days.

Ordinate Sulphaemoglobin concentration as percentage of total content of haemoglobin.

● PhN + Ph Phenacetin habitués with renal insufficiency during ingestion of phenacetin (B)

○ PhN + PPh Same patients during ingestion of phenacetin containing no acetic 4-chloranilid (C)

△ PhN — Ph Same patients without ingestion of phenacetin (A)

× Ph + Ph Phenacetin habitués without renal insufficiency during ingestion of phenacetin (D)

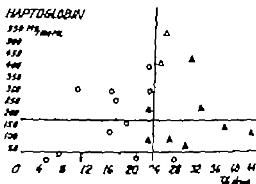


Fig 7 Relationship between erythrocyte biological half-lifetime and haptoglobin concentration in phenacetin habitués with renal insufficiency

Abscissa Biological half-lifetime in days.

Ordinate Haptoglobin concentration in mg/100 ml.

Symbols as in fig 5.

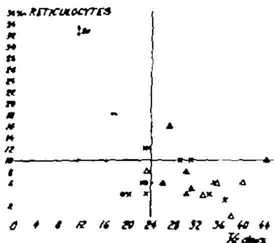


Fig 6 Relationship between erythrocyte biological half-lifetime and reticulocyte count in phenacetin habitués with renal insufficiency

Abscissa Biological half-lifetime in days.

Ordinat Number of reticulocytes as % of number of erythrocytes.

Symbols as in fig 5.

Discussion and conclusion

The present study has shown that the effect of phenacetin without acetic-4-chloranilide on the erythrocyte lifetime in phenacetin habitués with renal insufficiency is scarcely different from that of the ordinary phenacetin preparation containing that substance. As regards two patients (Nos 14 and 15) the lifetime values were normal during administration of the purified product but not when the impure phenacetin was given. This is not in agreement with the findings of Harvald et al (5) who reported that the urine sediment was increased by commercial phenacetin but not significantly by the purified product. If, as is the opinion of the writers, a sensitization mechanism is involved in the reduction of the erythrocyte lifetime — and there is much to support this theory (3 9) — this must be caused by the phenacetin as such and not to any extent by the acetic 4-chloranilide. This question will be investigated further at a later stage, using acetic-4-chloranilide alone

The fact that the sulphaemoglobin concentration tended to be greatest in the patients given phenacetin containing acetic-4-chloranilide would indicate that this impurity combined with the phenacetin may be of significance for the formation of sulphaemoglobin.

Summary

The effect of commercial phenacetin and a product without acetic-4-chloranilide on the erythrocyte survival time as determined by the chromium technique, the formation of sulphaemoglobin, the number of reticulocytes and the haptoglobin concentration, was examined on 15 phenacetin habitués with renal impairment. There was little definite difference between the two preparations in these respects. The biological half-lifetime of the erythrocytes was considerably reduced both by the administration of commercial phenacetin (from 31.2 to 15.6 days) and of phenacetin containing no acetic-4-chloranilide (from 31.2 to 18.0 days).

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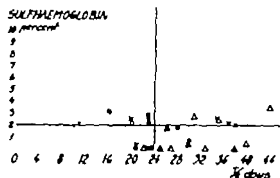


Fig. 5. Relationship between erythrocyte biological half lifetime and sulphaemoglobin concentration in phenacetin habitués with renal insufficiency.

Abscissa: Biological half-lifetime in days.

Ordinate: Sulphaemoglobin concentration as percentage of total content of haemoglobin.

● PhN + Ph: Phenacetin habitués with renal insufficiency during ingestion of phenacetin (B)

○ PhN + PPh: Same patients during ingestion of phenacetin containing no acetic-4-chloranilide (C)

△ PhN — Ph: Same patients without ingestion of phenacetin (A)

× Ph + Ph: Phenacetin habitués without renal insufficiency during ingestion of phenacetin (D)

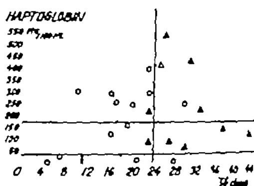


Fig. 7. Relationship between erythrocyte biological half-lifetime and haptoglobin concentration in phenacetin habitués with renal insufficiency.

Abscissa: Biological half-lifetime in days.

Ordinate: Haptoglobin concentration in mg/100 ml.

Symbols as in fig. 5.

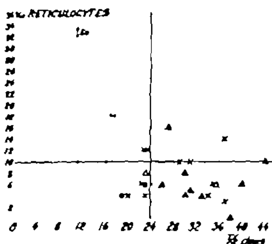


Fig. 6. Relationship between erythrocyte biological half-lifetime and reticulocyte count in phenacetin habitués with renal insufficiency.

Abscissa: Biological half-lifetime in days.

Ordinat: Number of reticulocytes as % of number of erythrocytes.

Symbols as in fig. 5.

Discussion and conclusion

The present study has shown that the effect of phenacetin without acetic-4-chloranilide on the erythrocyte lifetime in phenacetin habitués with renal insufficiency is scarcely different from that of the ordinary phenacetin preparation containing that substance. As regards two patients (Nos. 14 and 15) the lifetime values were normal during administration of the purified product but not when the impure phenacetin was given. This is not in agreement with the findings of Harvald et al. (5) who reported that the urine sediment was increased by commercial phenacetin but not significantly by the purified product. If, as is the opinion of the writers, a sensitization mechanism is involved in the reduction of the erythrocyte lifetime — and there is much to support this theory (3, 9) — this must be caused by the phenacetin as such and not to any extent by the acetic 4-chloranilide. This question will be investigated further at a later stage using acetic 4-chloranilide alone.

The fact that the sulphaemoglobin concentration tended to be greatest in the patients given phenacetin containing acetic-4-chloranilide would indicate that this impurity combined with the phenacetin may be of significance for the formation of sulphaemoglobin.

Summary

The effect of commercial phenacetin and a product without acetic-4-chloranilide on the erythrocyte survival time as determined by the chromium technique, the formation of sulphaemoglobin, the number of reticulocytes and the haptoglobin concentration, was examined on 15 phenacetin habitués with renal impairment. There was little definite difference between the two preparations in these respects. The biological half-lifetime of the erythrocytes was considerably reduced both by the administration of commercial phenacetin (from 31.2 to 15.6 days) and of phenacetin containing no acetic-4-chloranilide (from 31.2 to 18.0 days)

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Iodide Repletion Test in an Endemic Goitre Area

Risk of Iodine-induced Hyperthyroidism

By

Borje Ek, Sven Johnson and Bertil von Porat

In recent years two new valuable laboratory methods have been devised for demonstrating functional disorders of the thyroid gland, namely a tracer test with radioactive iodine (I^{131}) and, secondly determination of protein-bound iodine in the serum (PBI). These methods may sometimes, however give misleading values. In the presence of certain diseases, especially of the heart, liver and kidney both of the above-mentioned tests may give false results. The commonest cause of such erroneous values is, however probably previous treatment with iodine-containing medicine or examination with iodine-containing contrast medium.

Another important source of error in the I^{131}-test is the increased affinity of the thyroid for iodine in endemic goitre areas. In such cases the uptake of I^{131} by the thyroid is increased and the excretion in the urine is decreased — findings suggesting hyperthyroidism. Such observations have been made in Peru (7) Holland (1) Switzerland (3) and Finland (8) for example. The cause of these misleading results in the uptake and excretion of I^{131} is iodine deficiency. This may be primary and due to deficiency of iodine in the drinking water food etc., or secondary and due to the presence of substances (so-called goitrogens) preventing normal utilization of iodine.

In order to ascertain whether the increased uptake of I^{131} by the thyroid in such cases is due to "iodine deficiency" or hyperthyroidism, two tests have been used the triiodothyronine test (9) and the iodide repletion test (2).

In the former test the patient is given 100 μg of triiodothyronine a day for a week, by which time the value obtained by the I^{131}-test will become normal if the increase is caused by iodine deficiency but not if it is due to hyperthyroidism.

In the iodide repletion test a small amount of potassium iodide is given for a

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certain period after which the I^{131} uptake by the thyroid decreases in "iodine deficiency" but not in hyperthyroidism.

Burrel and Fraser (2) devised the following procedure The patient is given 10 mg of potassium iodide by mouth daily for two weeks. Four weeks after the last dose the I^{131} test is repeated In 24 of their 25 patients with slight hyperthyroidism the values found for the I^{131} uptake after this potassium iodide medication were still increased Of these patients thirteen received altogether 70 mg each eleven 140 mg and one 280 mg of iodide Of 38 patients with non toxic goitre showing avid thyroid uptake only one showed a somewhat increased uptake on repetition of the radioiodine test Seven of the patients were given altogether 70 mg of potassium iodide twenty six 140 mg and five 280 mg In 12 of these cases the uptake of I^{131} was abnormally low which suggested that the potassium iodide had impeded the uptake of the I^{131} The authors recommend the iodide repletion test when the simple I^{131} test reveals an unexpectedly avid thyroid uptake of dubious clinical significance

Radioiodine tests were introduced at Sundsvall hospital in 1957 The area served by the hospital is known as an endemic goitre area Despite the use of iodinized salt about every fourth patient admitted to the department of medicine has an enlarged thyroid gland During 1961 the I^{131} test gave increased values suggesting hyperthyroidism in 86 patients of these 35 were hyperthyroid while 51 (60 %) were euthyroid Of 256 euthyroid patients studied 51 (20 %) had values suggesting hyperthyroidism because of "iodine deficiency

As an example of these discrepancies, it might be mentioned that in a clinically euthyroid 42 year-old woman with neurovegetative symptoms the I^{131} test showed a 2 hour uptake of 77 % a 24-hour uptake of 92 % and a 24-hour urinary excretion of 1 % but the BMR was + 6 % and PBI 5 5 μg/100 ml.

In order to evaluate the iodide repletion test of Burrel and Fraser (2) in an endemic goitre area, we have used this test for about 2 years

Methods

I^{131}-test

The patient was given a tracer dose of I^{131} by mouth in the fasting state, and the uptake was measured over the thyroid 2 and 24 hours later (Th_2 and Th_{24} respectively) with a scintillation counter (10) The measuring distance was 50 cm and as a standard we used the same dose measured in a 100 ml graduated flask at the same distance The urine was collected for 24 hours (U_{24}) and measured against a standard

The normal values with our technique are Th_2 20—30 Th_{24} ?3—55 and U_{24} 30—60 %. These values were obtained from Larsson's (4) normal series after correction for the difference in the method regarding the measurement of the standard. The BMR was measured sometimes by the apparatus of Basal Jones, and otherwise by the apparatus of Krogh. The protein-bound iodine (PBI) was determined according to the method of Barker & Humphrey as modified by Skanse & Hedenskog (6) who also performed the analyses. The normal values are 3 8—8.0 μg (M $\pm$ 3 Σ)

Iodide repletion test

A pill containing 10 mg potassium iodide was given to the patient every day for 14 days. Four weeks after they had received the last pill the I^{131} test was repeated. In 104 cases the test was performed once in 12 twice and in one 3 times.

Material

The material consisted of 117 patients. In all of them the iodine uptake by the thyroid was increased and the excretion of iodine in the urine was decreased All the patients had

Table I Results of I^{131} -test before and after iodide repletion test (IRT) in the hyperthyroid group

	Before IRT (17 cases)		After IRT I (17 cases)		After IRT II (2 cases)	
	Mean value	Range	Mean value	Range	Mean value	Range
Th_4 ( )	54	40—77	41	36—59	45	35—56
Th_2 ( )	70	59—86	68	50—77	73	68—78
U_{24} ( )	18	8—29	21	6—28	23	17—29

Table II I^{131}-tests in which simple iodide repletion test (IRT) did not normalize the I^{131}-test although the patients were euthyroid

Case	Th_4 ( ) Before	IRT I	IRT II	Th_4 ( ) Before	IRT I	IRT II	U ( ) Before	IRT I	IRT II
1	55	39	24	77	57	30	90	38	39
2	77	34	17	92	64	39	1	27	45
3	64	43	30	79	63	45	22	36	32
4	51	42	18	79	73	31	17	30	51
5	40	30	27	63	60	30	13	37	51
6	83	43	23	100	66	38	6	37	44

been admitted to the medical department and examined clinically within 2—4 days of admission, by one of us (S. J I 100 of these cases clinical examination and laboratory findings (BMR and PBI definitely have shown that they were euthyroid at the moment of the first examination. The remaining 17 patients had moderate clinical hyperthyroidism I all of these patients the BMR was increased (average — 45 range between + 17 and + 89) as was the PBI value (average 12.5 µg range between 9.2 and 18.5 µg 100 ml

I the euthyroid group as well as in the hyperthyroid group the cases were specially selected in such a way that clinical examination and laboratory findings agreed.

The euthyroid group consisted of 87 females and 13 males, aged 17 to 72 years the hyperthyroid group, of 15 females and 2 males, aged 41 to 76 years.

Table III. I^{131} -tests in the 2 patients with exophthalmos or nephrosis, where the 2 iodide repletion tests (IRT) performed three and two times respectively did not normalize the I^{131} -test

	Th_4 ( )	Th_2 ( )	U (%)
Exophthalmos			
Before IRT	60	63	21
IRT I	39	64	18
IRT II	37	62	23
IRT III	38	63	22
Nephrosis			
Before IRT	47	74	9
IRT I	44	78	10
IRT II	40	63	10

Results

A. Hyperthyroid group (17 cases)

The I^{131}-test still gave hyperthyroid values, though the Th_4- and Th_{24}-values were sometimes slightly lower than before the iodine medication. In 2 cases the iodide repletion test was repeated, but despite 2 periods of treatment with potassium iodide the I^{131}-values did not become normal. In none of these cases was any clinical impairment observed

Table IV Survey of clinical and laboratory findings in the 7 patients who developed hyperthyroidism afte the iodide repletion test (IRT)

Case	Test	Age	Sex	Thyroid enlargement	Clinical state	BMR (%)	PBI (µg%)	Th_2 (%)	Th_{24} (%)	U_{24} (%)
1	Before	34	♀	Moderate	Euthyr	+19	5.8	50	67	14
	IRT I				Uncertain	—	—	44	60	25
	IRT II				Thyrotoxic	—	10.2	51	63	13
2	Before	63	♀	Moderate	Euthyr	+27	6.6	56	79	16
	IRT I				Thyrotoxic	—	11.6	37	69	15
3	Before	61	♀	Slight	Euthyr	—	3.6	53	73	18
	IRT I				Thyrotoxic	+89	16.3	59	72	6
4	Before	68	♀	Moderate	Euthyr	+14	5.0	64	78	10
	IRT I				Euthyr	—	10.4	34	76	13
	IRT II				Thyrotoxic	—	10.8	56	78	20
5	Before	40	♀	Moderate	Euthyr	− 1	5.6	40	68	33
	IRT I				Uncertain	—	—	37	73	24
	IRT II				Thyrotoxic	+81	24.5	63	83	4
6	Before	51	♀	Moderate	Euthyr	+35	6.2	46	73	28
	IRT I				Euthyr	—	11.6	47	68	24
	IRT II				Thyrotoxic	—	10.8	43	63	23
7	Before	68	♀	Moderate	Euthyr	± 0	8.0	48	81	12
	IRT I				Thyrotoxic	+16	17 7	34	73	18

after the test The results of the I^{131} test before and after the iodide repletion test are compared in table I

The largest decreases in the Th_2 and Th_{24} values were from 70 % to 44 and from 82 % to 50 % respectively and the largest increase in U_2 was from 8 % to 26 %

B Euthyroid group (100 cases)

The results of the iodide repletion tests in these patients fall into 4 groups.

I Normalization of the I^{131}-test (58 cases)

II Hypothyroid values (blockage) (33 cases)

III Unchanged hyperthyroid values despite euthyroidism (2 cases)

IV Unchanged hyperthyroid values and development of hyperthyroidism as judged by clinical and laboratory findings (7 cases)

In groups I—III the clinical picture was the same after the test as before. No substantial increase in the PBI values occurred. Before the test the mean value for the group was 5.6 µg/100 ml (range 3.1—8 1 µg/100 ml) and afterwards the mean value was 6 3 µg/100 ml (range 4—8.8 µg/100 ml)

In 6 patients of group I the values obtained in the I^{131}-test did not become normal until after a second iodide repletion test. The values before, after the first, and after the second repletion test are given in table II

All of these 6 patients showed a reduction of Th_2 as well as Th_2 after the first repletion test but not until after the second test were both values normal,

though U_{24} was normalized in five patients already after the first test.

Of the 2 patients (group III) in whom the values did not become normal after 3 and 2 tests, respectively but who were still euthyroid one had exophthalmos (3 tests) and the other nephrosis (2 tests) The results of the I^{131} test in these 2 patients are given in table III

The most interesting group is that consisting of the 7 patients who after having received 140 mg (3 cases) or 280 mg of potassium iodide (4 cases) became hyperthyroid Clinical and laboratory data on these cases are given in table IV All these seven patients have been treated with I^{131} The doses and the end results of treatment are given in table V In 3 of these patients treatment with I^{131} was complicated. In 2 (Nos. 3 and 7) the first I^{131}-treatment was followed by considerable clinical deterioration with threatening hyperthyroid crisis. Both were treated with large doses of iodine and antithyroid drugs. The last patient is still under treatment. In one (No. 5) I^{131}-treatment was followed by a persistent hypothyroidism requiring substitution therapy

Table V Results of treatment in the 7 patients with iodine-induced hyperthyroidism

Case	Treatment I^{131} (μC)	PBI (μg %)	I^{131} (%)		
			Th_1	Th_2	U_t
1	5	4 7	44	57	29
2	6	7.3	26	43	35
3	6+3+5 + propylthiouracil	7.2	42	55	16
4	6+4	5.6	23	58	27
5	4+4	2.3	23	25	52
6	5	7.6	28	42	49
7	5+ carbimazol	Treatment with carbimazol in progress Last PBI value 5.6 μg %			

Discussion

In the investigation of thyroid function with the I^{131}-test in an endemic goitre area the test gave results suggesting hyperthyroidism in about 20 per cent of euthyroid patients. A hyperthyroid result was more frequently due to "iodine deficiency" than to hyperthyroidism. This shows that the patient history and the clinical findings must be borne in mind in the interpretation of laboratory tests.

In the differentiation between iodine deficiency" and hyperthyroidism we tried Burrel and Fraser s (2) iodid repletion test. Like Burrel and Fraser we found that the increased uptake of I^{131} by the thyroid did not diminish to normal after the test in the presence of hyperthyroidism.

In the euthyroid patients the values obtained in the I^{131}-test became normal (58 %) or decreased to levels suggesting hypothyroidism or blockage. The latter result was obtained in 33 % of the cases or just as often as in Burrel and Fraser s series. This response to the iodide repletion test is not misleading, because normalization or depression of the values to subnormal levels in our investigation has always signified that the patient was euthyroid.

A disadvantage of the test, however was that in 6 euthyroid patients the iodine uptake by the thyroid did not become normal, though the excretion of I^{131} in the urine increased to normal values in 5 of these patients. In these cases, the test did not decide with certainty whether the patients had "iodine deficiency" or hyperthyroidism. After a second iodide re-

pletion test the I^{131} test showed a normal or a decreased uptake of I^{131} by the thyroid and normal excretion in the urine in all 6 cases. The reason why these 6 patients required two periods of treatment with potassium iodide was probably a marked iodine deficiency, *i.e.* the first 140 mg of potassium iodide was not sufficient to depress the avidity of the thyroid for iodine. In such cases it thus requires about 3 months before it is possible to distinguish between "iodine deficiency" and hyperthyroidism.

In 2 euthyroid patients the I^{131} values did not become normal even after 2 and 3 repletion tests, respectively. One of them had nephrosis, and the probable reason why the I^{131}-test did not become normal after two repletion tests is that the thyroid produces an increased amount of hormone, which is partly lost in the urine (5). The other patient had exophthalmos. She was first given 2 tests with potassium iodide in succession and after 2 years a further test without any change in the I^{131} values. Burrel and Fraser (2) also found that the I^{131} values failed to become normal in 2 of 3 euthyroid patients with exophthalmos in their series. This negative result is probably due to a markedly increased TSH production which prevents reduction of the iodine uptake.

In patients with exophthalmos or nephrosis, then, persistently increased uptake of I^{131} by the thyroid after the repletion iodide test does not necessarily mean that the patient is hyperthyroid.

The most serious objection to the iodide repletion test is, however, that it may produce hyperthyroidism in patients in an endemic goitre area. Burrel and Fraser (2) have discussed the possibility of iodine-induced hyperthyroidism but they have so far not encountered this complication after some 200 tests. Stanbury et al. (7) report a case in which treatment with 1.5 mg of iodide a day for 2 to 3 months was followed by the development of hyperthyroidism. Burrel and Fraser ascribe the absence of iodine-induced hyperthyroidism in their series to the fact that they used a higher iodide dosage for a shorter period.

In as many as 7 (7 %) of our euthyroid patients, however, clinical examination and laboratory studies showed characteristic hyperthyroidism immediately after the iodide repletion test. They were certainly euthyroid before the test, when they had shown no clinical evidence of hyperthyroidism, and in all of them the PBI was normal (case 7 had a value bordering the upper normal limit). In cases 2 and 6 the BMR was somewhat high (+ 27 and + 35 %) but the examinations were performed with a Basal Jones apparatus, which often gives a false high value. That the patients were hyperthyroid after the test may also be regarded as certain. They all showed clinical evidence of fairly advanced hyperthyroidism. All of them had a persistent high uptake of I^{131} by the thyroid and in all of them the PBI values were abnormally high. The administration of potassium iodide cannot explain the increased PBI value, because no increase was noted among the 93 patients who were still euthyroid.

The possibility that these patients would have developed hyperthyroidism even if they had not been examined with the iodide repletion test must, in our opinion, be rejected. *If only one* of the patients had become hyperthyroid this possibility would have to be considered, but since hyperthyroidism developed in as many as 7 per cent of the patients immediately after the test, the

complication must be ascribed to the administration of iodide.

In 4 of these 7 cases the tests were performed twice, because the uptake of iodine was not normalized after th first test dose. Two (Nos. 1 and 5) of these cases were judged clinically as uncertain, is small changes had occurred in the clinical picture, which made the diagnosis of euthyroidism uncertain. Unfortunately no PBI-determinations were mad at that time. The other 2 patients, who were tested twice were judged clinically as euthyroid after the first test but later after the second test had been started, PBI determined after the first iodide repletion test showed pathologically increased value (case 4 increased from 5 to 10.4 µg case 6 increased from 6.2 to 11.6 µg). It is thus possible that even a single iodide epletion test would have been sufficient to induce hyperthyroidism in these 4 patients.

We believe that these 7 patients had a hyperfunctioning thyro d but their iodine deficiency has prevented the production of sufficient thyroid hormone to develop hyperthyroidism. After the administration of potassium iodide they had sufficient iodine to produce thyroid hormone n amounts large enough to cause symptoms of hyperthyroidism The risk of iodine induced hyperthyroidism is, in our opinion, greatest in endemic goitr areas, with iodine deficiency

It would have been ery interesting to have left these cases untreated to ascertain whether the picture of hyperthyroidism gradually disappeared spontaneously This was prohibited on ethical grounds, especially since w had induced the condition by our examination methods and since all had rather severe symptoms.

In view of the high frequency of iodin induced hyperthyroidism we consider the use of the iodide repletion test unsuitable and even dangerous in the d fferential diagnosis between iodine deficiency" and hyperthyroidism We have therfore abandoned this test and now use a deter mination of PBI[131] for this purpose

Another disadvantage of the iodide repletion test is that it requires 6 weeks or even 12 weeks (if a second test is necessary) before differentiation is possible.

Our nvestigation shows that in endemic goitre areas the administration of iodine involves the risk of development of hyperthyroidism

Summary

The iodide repletion test of Burrel and Fraser was used in n endemic goitre area, where the I^{131} test often gives false high values because of iodine deficiency" The patients were given 10 mg potassium iodide daily for 2 weeks. Four weeks after the last dose the I^{131}-test was repeated

The test was performed on 17 patients with hyperthyroidism and 100 euthyroid patients n whom the values obtained with the I^{131}-test suggested hyperthyroidism.

In all 17 patients with hyperthyroidism the values obtained n the second I^{131} test were not normalized and the test were not followed by any complications.

In 58 of the 100 euthyroid patients the I^{131} values became normal after one (52) or two (6) iodide repletion tests. In 33 patients the values obtained after the test indicated hypothyroidism. In 2 patients, one with nephrosis and one with exophthalmos, the I^{131}-values remained abnormal even after 2 and 3 repletion tests, respectively In as many as 7 cases clinical hyperthyroidism, confirmed by laboratory studies, developed after the test.

We therefore believe that the use of the iodide repletion test is unsuitable and hazardous in the differential diagnosis between iodine deficiency and hyperthyroidism. The investigations also show that the risk of iodine-induced hyperthyroidism must be borne in mind in endemic goitre areas.

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Comparison of two Spironolactone Preparations in the Long-term Treatment of Oedematous Heart Failure

By

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In a previous paper we have demonstrated the favourable effect obtained by a supplement of spironolactone in cases of refractory cardiac failure on continuous treatment with the benzothiadiazine compound cyclopenthiazide. Spironolactone did not only prevent hypokalaemia and metabolic baseosis during the benzothiadiazine therapy but also entailed a considerable extra weight loss in several patients (1). The drug used was the commercially available spironolactone (Aldactone®) administered in the generally recommended dose of 400 mg daily.

Since spironolactone is expensive, efforts have been made to potentiate its effect, primarily by improving its absorption from the intestinal tract. Most recently it has been marketed in a preparation containing the spironolactone in a particle size of about 50 μm (micronized spironolactone). This is the preparation marketed to-day as Aldactone®. The recommended dosage is 100 mg daily.

During the past 6 months we have had occasion to compare the therapeutic effect of the older and the new spironolactone preparations, in the following designated as spironolactone and micronized spironolactone respectively.

Material and method

The series comprises 17 patients with oedematous heart failure who had failed to give satisfactory response to cyclopenthiazide (Navidrex®). While still on the cyclopenthiazide medication (1 mg daily), they received a supplement of spironolactone 400 mg daily or micronized spironolactone 100 mg daily in varying sequence through a period of one month.

The effect of this supplementary medication was assessed on the basis of alterations in body weight and in serum electrolytes.

The chemical analyses were performed in the Central Laboratory using methods described in a previous paper (2).

Results

Table I shows that the spironolactone supplement entailed a considerable weight loss in the entire group and that the body weight was the same on both regimes.

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Table I Mean body weight of 17 patients with oedematous heart failure during treatment with cyclopenthiazide 1 mg daily with or without a supplement of spironolactone

	Supplement of		No supplement
	Spironolactone 400 mg daily for 1 month	Micronized spironolactone 100 mg daily for 1 month	
Mean body weight (kg)	62.5	62.2	66.0

Table II Mean serum electrolyte values in 17 patients with oedematous heart failure during treatment with cyclopenthiazide 1 mg daily and a supplement of spironolactone

Serum electrolytes	Supplement of	
	Spironolactone 400 mg daily for 1 month	Micronized spironolactone 100 mg daily for 1 month
Serum sodium	137.9 mEq/l	137.5 mEq/l
Serum potassium	4.32 mEq/l	4.45 mEq/l
Serum chloride	94.5 mEq/l	96.4 mEq/l
Standard bicarbonate	23.6 mEq/l	23.8 mEq/l
pH	7.41	7.41
pCO	39.9 mm Hg	39.0 mm Hg

As apparent from table II the serum electrolyte patterns were exactly identical in the two series. It will be noted that serum potassium and standard bicarbonate remained normal while the serum chloride concentration was reduced in both groups.

Discussion and conclusion

The experiments show that 100 mg spironolactone in micronized form exerts the same effect on body weight and serum electrolyte pattern as 400 mg spironolactone of the older preparation when used in combination with cyclopenthiazide in refractory heart failure.

Since in our experience there is a certain proportionality between the effect and the dosage of spironolactone in refractory cardiac oedema, the results indicate that micronized spironolactone, per weight unit, is about 4 times as active as the earlier preparation of the drug.

Summary

The effect of two spironolactone preparations was compared in 17 patients with refractory cardiac failure on continuous treatment with cyclopenthiazide 1 mg daily. For a period of one month the patients received, in varying sequence, a supplement of the older type of spironolactone 400 mg daily or the new micronized spironolactone 100 mg daily.

The two forms of treatment entailed the same weight loss and counteracted to the same extent the development of hypokalaemia and metabolic baseosis during cyclopenthiazide therapy. It is concluded that in the present series 100 mg micronized spironolactone proved equally effective as 400 mg of the older type of spironolactone.

Acknowledgement

Our thanks are due to Mr. P. Amers of Messrs. Searle who supplied us with spironolactone (Aldactone D) for these experiments.

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From the Department of Medicine (Head: Erik Ask-Upmark, M. D.) University Hospital, Uppsala, Sweden

Basophil Leukocytes in Ulcerative Colitis

By

Lennart Juhlin

The etiology of ulcerative colitis is still under discussion. Allergic, bacterial, enzymatic and psychogenic factors have been mentioned in the abundant literature on this subject (1, 5, 9, 21, 23).

A new concept of the problem was offered by McGovern and Archer (14). They found an increased number of mast cells around the vessels which penetrate the inner circular coat of the submucosa in patients with ulcerative colitis. These findings were confirmed by McAuley and Sommers (12). The mast cells contain histamine, heparin, and proteolytic enzymes (11) which, when released, could give rise to the pathological changes found in the disease. Thus, liberated histamine was considered to cause vascular congestion and a spastic state of the colon musculature. This continuous contraction of the muscle, together with the congestion and edema of the mucosa and submucosa, would convert the colon into a thick-walled semi-rigid tube which no longer contracted segmentally and which lacked haustra (14). The relative absence of inflammatory cells in the submucosa prior to ulceration supports this interpretation. The superficial ulceration was thought to be due to simple excoriation. The authors concluded that ulcerative colitis was within the realm of psychosomatic disorders and added that nervous impulses induced excessive production of acetylcholine in the colon. According to McGovern, acetylcholine is a powerful histamine-releasing agent. However, "psychosomatic" factors need not be involved in incorporating the mast cells in theories concerning the pathogenesis of ulcerative colitis (10). A release of histamine from the mast cells can also be produced by allergic and enzymatic factors. The findings of Mirvich (15) are of interest in this connection. He observed that mechanical *stimulation of the* normal-looking mucosa in ulcerative colitis led to the development of a localized area of edema resembling the "triple response" of the skin.

There is a "cousin cell" of the mast cell in the bloodstream, namely the basophil leukocyte, which is known to contain heparin (2) and most of the circulating

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histamine (6). Priest et al. (16) observed an increased amount of basophils in artificially produced skin lesions in patients with ulcerative colitis. The increased amount of basophils found with the "skin window technique" was not due to an increase of the cells in the blood. For example, a patient with myelogenous leukemia with a high blood basophil level (37 %) did not show this inflammatory response (17). The findings suggest that the basophil leukocyte might be a cell involved in the mechanism causing ulcerative colitis.

A new method for dealing with the basophil leukocytes has recently been described (19). The basophils are divided into 20 different types according to size, staining properties and location of the basophil granules. Using this method we have studied the basophils of the blood, experimentally produced skin lesions, rectal secretions and superficial suction biopsies of the affected mucosa. The number of basophils/mm³ blood has been compared with that of the eosinophils. In a few patients, the percentage of basophils in the bone marrow was examined simultaneously.

Material

Ten patients (aged 20—65 years) having acute exacerbation of ulcerative colitis with frequent bloody stools were studied 5—8 days after admittance to the hospital. Their general condition was fairly good. The hemoglobin values were between 70—95 (10.6—14.4 mg%). The patients were kept in bed most of the day and given a protein-rich diet described by Ask Upmark (4). They were treated with salicylazosulfapyridine (Salazopyrin®), spasmolytics, sedatives and vitamins. When their disease had improved, further specimens were taken for basophil examination.

Methods

Counting and examination of basophils in blood

The method for handling the basophils has been described elsewhere in detail (19). In outline, venous blood is drawn into a siliconized needle and syringe and forcibly ejected into a cold fixative (acetic acid 20, ethyl alcohol 60, chloroform 20). This destroys all of the erythrocytes, and gives an immediate fixation of the leukocytes. The white cell suspension is filtered through a coarse membrane filter of cellulose (Cella 0). The cells remain on the filter where they are stained with toluidine blue. The filter is then dehydrated in ethyl alcohol, cleared in xylene, and mounted a microscope slide. It is then possible to study the morphology of the basophils, which appear as distinct cells with specific dark metachromatic staining of the granules. The other white cells are pale blue with a faint greenish tint in the granules of the eosinophils.

Under a magnification of 640 × 40 consecutive basophils were classified into three major groups A, B and C, depending on the size of the granules and the depth of the staining. Furthermore, each group is subdivided into six classes (1 to 6) reflecting the number and location of the granules.

The percentage of basophils was estimated by counting the number of all the white cells seen while finding the 40 basophils. The total number of basophils has been estimated by simultaneously counting the number of white cells/mm³.

Counting of basophils in bone marrow

Bone marrow specimens were taken from three patients only. They were treated essentially as the blood specimens. For details of the method see Juhlin (7).

Examination of basophil leukocytes in blisters of the skin

Skin inflammation was produced on the inside of the forearm by applying cantharidin in a waxy base (Emplastrum cantharidin c euphorb, Swedish MB 37). It had the following composition: Terpentina laricina 6, mastic 6, euphorbia 1 and cantharis 2. A piece of cantharidin (1 cm square and about 1 mm thick) was applied with elastic adhesive plaster and allowed to remain for 20—24 hours.

When removing the plaster care was taken not to destroy the blister formed. When no blister was obtained, the procedure was repeated. The whole content of the blister (0.05—0.3 ml) was aspirated with a siliconized needle and syringe and added to 10 ml of the fixative. It was then treated as described for blood.

Rectal secretion and biopsy specimens

The patients were asked to try to evacuate the rectum before rectoscopy. No laxatives or clysters were given. During rectoscopy secretion was taken from the affected mucosa with a piece of polyethylene tubing. Two to three drops were added to 10 ml of the fixative and stain. W always tried to get tw types of specimens, one clear and the other slightly bloody. The "suction biopsy" was done by scraping the affected mucosa with the obliquely cut piece of tubing and at the same time applying suction with syringe coupled to the other end of the tubing. The biopsy thus obtained contained only the most superficial part of the mucosa. It was put into the fixative and treated as described above.

Eosinophils

The total number of eosinophils was counted in the manner described by Thorn et al. (22).

Results

1. The number of basophil leukocytes per mm³ of blood was increased in the acute stage of the disease (fig. 1 and table I). The percentage of basophils, however, was the same as in normal subjects due to an increase in the total leukocyte count. Compared to patients with the same degree of leukocytosis, the percentage of basophils in ulcerativ colitis was increased. In patients with ulcerative colitis the number of melted cells (group A) was higher by 13 %, which was probably significant ($P < 0.05$). The sum of the cells B_1, B_2, C_1 and C_2 was decreased ($P < 0.01$). Otherwise the basophil differential was similar to that of normal

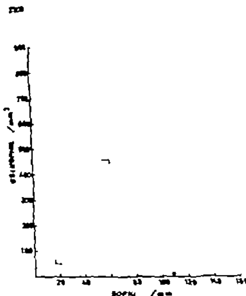


Fig. 1. The number of basophil and eosinophil leukocytes in blood from patients with ulcerative colitis. The number of these cells from 22 healthy subjects fell within the range represented by the rectangular area.

subjects and showed no signs of increased degranulation (table II). When the patients condition had improved the total basophil and white cell count became normal.

2. As is evident from fig. 1 some of the patients had a high eosinophil leukocyte count during exacerbation. Values approaching normal were restored during treatment.

3. The cantharidin blisters contained between 0.06—1.07 per cent basophils (mean value 0.38 %). The number and type of basophils was the same as found in normal subjects (table III). No correlation was found between the number of basophils in blood and blisters (fig. 2). The blister basophils were more degranulated (types A_{4-5}, B_{4-5} and C_1) and also more melted (types A_{1-3}) than blood basophils.

histamine (6) Priest et al (16) observed an increased amount of basophils in artificially produced skin lesions in patients with ulcerative colitis. The increased amount of basophils found with the "skin window technique was not due to an increase of the cells in the blood For example, a patient with myelogenous leukemia with a high blood basophil level (37 %) did not show this inflammatory response (17) The findings suggest that the basophil leukocyte might be a cell involved in the mechanism causing ulcerative colitis.

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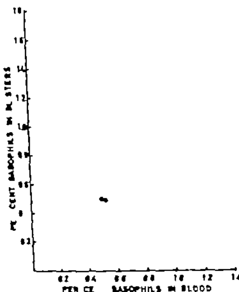


Fig. 2. The percentage of basophil leukocytes in blood and cantharidin blisters.

○ Healthy subjects.

● Ulcerative colitis.

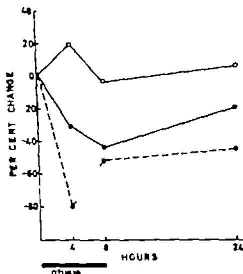


Fig. 3. The effect of an 8-hour intravenous infusion of corticotrophin (25 IU) and saline in an ulcerative colitis patient lacking blood eosinophils.

● – – ● ACTH infusion in acute stage of the disease. Initial number of basophils 110/mm³

○——○ Saline infusion one week later. Initial number of basophils 82/mm³

●——● ACTH infusion when the patients condition had improved. Initial number of basophils 48/mm³

4. The "clear" rectal secretion contained between 0.001—0.2 per cent (mean 0.1 %) basophils in the acute stage of the disease. They were all degranulated with only a few residual granules (group 6) of which about half were melted (type A_4). In the secretion, which was slightly blood-colored, the same types of cells were seen. The percentage of basophils was in the latter slightly decreased (mean 0.06 %). The number of basophils in different specimens from the same individual varied. The most common white cell in both the clear and the bloody secretions was the polymorphonuclear leukocyte, which often contained bacteria. Eosinophils were fairly common, but the method does not allow any exact calculation of the number of these cells.

All of the specimens taken from the superficial mucosa by suction showed a massive infiltration with white cells, with a dominance of polymorphonuclear leukocytes. The percentage of basophils was difficult to estimate, but an approximate mean value would be 0.01 per cent. Most of the basophils seen were non-degranulated (type B_1). No cells which could be identified as mast cells were found.

5. In five of the patients, the rectal secretion was examined when the condition had improved. The amount of basophils was between 0.001—0.01 per cent, all of which were degranulated. Mononuclear cells, mainly lymphocytes, were now more common than in the acute stage.

6. The number of basophils in bone marrow was examined in 3 patients. It contained 0.25, 0.35 and 0.50 % baso-

Table I The mean number and range of basophils, eosinophils and total white blood cells in patients with ulcerative colitis and various controls

	No. of subjects	White blood cells per mm³	Eosinophils per mm	Basophils per mm³
Ulcerative colitis	10	11,500 (7 400—19 000)	592 (0—2,500)	74 (38—151)
Healthy subjects	22	5,100 (4,000—8,900)	162 (50—470)	33 (16—61)
Myocardial infarction	10	11,000 (7 100—19 400)	40 (0—280)	20 (1—60)
Acute infections	10	13,900 (10,000—15 100)	64 (0—260)	19 (1—48)
Terminal ileitis	2	9,500, 10 700	78, 28	24 11
Regional colitis (Mb. Crohn)	1	14,900	439	43
Non-ulcerative colitis	1	10,800	0	10
Malabsorption	2	3,600 7,900	56 0	10 10

Table II The mean blood basophil differential in 10 subjects with ulcerative colitis in the acute stage For comparison the mean blood basophil differential in 22 healthy subjects is shown within brackets

Class	Group A "Melted" clumps of granules	Group B Discrete medium granules	Group C Small faint granules
1 Numerous intracellular	5 (2)	4 (11)	5 (10)
2. Ring aggregation	10 (9)	9 (12)	3 (6)
3. Surface extrusion	9 (7)	8 (6)	2 (2)
4 Extracellular spray	8 (4)	5 (5)	2 (1)
5 Massive release	4 (1)	8 (2)	2 (1)
6. Residual few	8 (7)	6 (7)	2 (6)

Table III The mean basophil differential in cantharidin blisters from 9 patients with ulcerative colitis The mean basophil differential in cantharidin blisters from 15 normal subjects is shown within brackets

Class	Group A "Melted" clumps of granules	Group B Discrete medium granules	Group C Small faint granules
1 Numerous intracellular	10 (9)	5 (4)	1 (1)
2. Ring aggregation	26 (17)	4 (2)	1 (2)
3. Surface extrusion	8 (11)	0 (2)	1 (1)
4 Extracellular spray	3 (5)	1 (1)	0 (1)
5. Massive release	1 (4)	0 (2)	0 (1)
6. Residual few	20 (28)	13 (8)	6 (1)

resistant to corticosteroids or stress situations. This, however is not the case since this patient showed a marked decrease in the number of basophils during intravenous infusion of corticotrophin.

The increased number of blood basophils in patients with ulcerative colitis can be considered as a characteristic for these patients. It cannot in itself be an explanation for their symptoms since these are lacking in many other patients with basophilia. Priest et al. (16) observed that the inflammatory response was altered in artificially produced skin lesions. They found an increase of basophil leukocytes in the skin lesions of 13 out of 19 patients with ulcerative colitis. The method used was the "skin window" technique, where the epithelium was scraped away diphtheria toxoid applied and the lesion covered with a cover slip Such a selective increase of basophils in an inflammatory area could explain the pathological changes of the colon in the same way as proposed by McGovern and Archer (14) for the mast cells. In my investigation, skin lesions were obtained by producing cantharidin blisters. The percentage of basophil leukocytes in these blisters was not increased in patients with ulcerative colitis. Several of the basophils were degranulated and of a melted type. Such a melting effect has earlier been found when Lecithinase A was added to the cells in vitro (20) In higher concentrations, Lecithinase A caused a complete lysis of the cells Therefore one possibility for explaining the discrepancy between my results and those of Priest et al. (16) could be that cantharidin destroys the surplus of basophils. However this does not seem likely since, in several other patients, as much as 2—10 per cent basophils has been found in the blisters. Such high values have, for example, been found in repeated examinations of blisters in 2 patients with myelofibrosclerosis. These patients also had an increased number of basophils in the bone marrow but slightly decreased values in the blood stream. Further studies of the basophils in differently produced inflammations therefore seems indicated.

McAuley and Sommers (12) as well as Anthonisen and Riis (3) found no basophils in rectal secretions from patients with ulcerative colitis. In the present investigation the percentage of basophils found in rectal secretions and superficial mucosal biopsies was very low The basophils in the secretions were all degranulated with only a few residual granules. Thus the possibility exists that there could have been an increased amount of basophils in the inflamed mucosa, which, due to complete basophil destruction, *no* longer was visible. However the fact that there is evidently *no* increase in basophils in the superficial mucosa, and that those which may be seen are mostly non degranulated cells, contradicts this theory A certain number of basophils are, however resistant to histamine liberators (20) If these resistant cells are the ones observed in the superficial mucosal layers, a complete basophil degranulation could have taken place in the deeper layers. One must then assume that the cells can be destroyed when they come out into the "free" rectal secretion. Such an explanation might seem far fetched, but I think the possibility must be left open.

Summary

The number and morphology of the basophil leukocytes have been investigated in 10 patients with ulcerative

phils, which is within normal limits. One of these patients had no eosinophils in the blood during an observation period of 5 months. His bone marrow contained 2 % eosinophils.

7 In the patient lacking blood eosinophils, the reaction of the basophils to intravenous infusions of ACTH (25 IU) in saline solution during 8 hours was studied both in the acute stage and when the condition had improved The decrease of the basophils was somewhat more pronounced than is usually seen (fig 3) The increase in 17 keto and 17 hydroxy steroids in the urine after the ACTH test was normal.

Discussion

The number of basophils per mm³ was significantly increased (74/mm³) in patients with ulcerative colitis in the acute stage as compared to normal subjects (35/mm) The percentage of basophils, however was the same (0 64 %) as in normal subjects due to an increase in the total white cell count. To determine if the increase in blood basophil leukocytes is specific for patients with ulcerative colitis, a comparison must be made with basophil counts on blood of subjects with an increased leukocyte count. A group of 10 patients with myocardial infarction was followed with blood basophil determinations for some days after the onset of the disease. The first few days after the heart attack, the leukocyte count ranged between 7 100—19 400 and then gradually decreased toward normal values. The number of basophils per mm³ blood was decreased (mean value 20/mm) as long as the leukocyte count remained elevated Therefore, there was a still more marked decrease in the basophil percentage. A similar decrease in the number of basophils associated with leukocytosis was found in patients with various infections, terminal ileitis and non-ulcerative colitis (table I) Among over 300 patients with various diseases where the total number of basophils has been determined an increase in the basophil percentage together with an increased leukocyte count has been found only in patients with erythrodermia, polycythemia and chronic myelogenous leukemia The increase of basophil leukocytes in patients with ulcerative colitis must therefore be considered as real.

Stress situations and corticosteroids are known to decrease the number of basophil leukocytes (8) The decrease in basophils after a myocardial infarction or an infection could thus be explained. It seems, however remarkable that patients with the frequent bloody diarrheas do not react in the same way It can be argued that the degree of stress is less in such cases. However we did not consider that the patients with regional ileitis or non-ulcerative colitis were subjected to more stress than those with ulcerative colitis.

Rasager (18) showed that eosinophil leukocytes increased in the blood during relapse of ulcerative colitis. Of the patients in our investigation some had very high eosinophil values. Others had normal values or no eosinophils. No relationship was found between the amount of eosinophils and basophils. The number of eosinophil leukocytes can therefore not be used as a measure of the degree of stress in patients with ulcerative colitis.

The eosinophils in patients with ulcerative colitis usually show a normal decrease to ACTH in the Thorn's test (5) In a patient with no eosinophils but with a high number of basophils, one might assume that the basophils were

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colitis. Specimens were taken simultaneously from blood bone marrow cantharidin skin blisters, rectal secretion and superficial biopsies from the affected rectal mucosa. Patients in the acute stage of ulcerative colitis had an increased amount of blood basophils (74/mm³) as compared to normal subjects (35/mm³) Due to an increase in the total leukocyte count in ulcerative colitis, the percentage of basophils was the same as in normal subjects The basophil percentage in ulcerative colitis was, however significantly increased when compared to other patients with a similar degree of leukocytosis. The blood basophils probably contained more ($P < 0\ 05$) melted or fused granules in ulcerative colitis than in normal subjects and other patients used as controls. No correlation was found between the number of basophils and eosinophils in blood Cantharidin-produced blisters of the skin contained 0 06—1 07 per cent basophils, which was the same as found in normal subjects. The basophils in the blisters were more degranulated than in blood and were often of the melted type. No correlation was found between the number of basophils in blisters and that in blood In the acute stage of colitis, the rectal secretion contained between 0 001—0 2 per cent basophils, all of which were degranulated with only a few residual granules. The number of basophils was similar in specimens from the superficial mucosa but in these specimens the cells usually were non-degranulated

No mast cells were found. The increase in basophil and eosinophil leukocytes subsided when the patients condition improved. The possible role of the basophil leukocytes in the mechanism causing the pathological changes in ulcerative colitis has been discussed

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Metabolism of Tryptophan in Diabetes Mellitus

By

Martti Oka and V. V. E. Leppänen

Some previous studies indicate that diabetes mellitus is one of those diseases in which a disorder of tryptophan metabolism is present. Rosen et al. (7) found that diabetic patients excreted on the average significantly greater quantities of xanthurenic acid after an oral test load of 10 g of DL-tryptophan than did non-diabetic controls. Kotake and Tani (3) detected xanthurenic and 3-hydroxykynurenic acids in the urine of diabetics, while paper chromatography did not reveal these substances in that of normal subjects. Conflicting results were obtained, however by another group of workers (10). Their diabetic subjects were found to excrete a subnormal proportion of the administered load of 4 g of L-tryptophan in the form of tryptophan, kynurenine anthranilic acid and xanthurenic acid, but normal amounts of 5-hydroxyindoleacetic acid.

According to a paper chromatographic study by the authors (6) tryptophan metabolism is deranged in diabetes mellitus. Eleven out of 23 diabetics showed abnormal indole chromatograms. Deviations from the normal occurred irrespective of the treatment the patients had received.

In this investigation an attempt has been made further to clarify the metabolic pattern of tryptophan in diabetes mellitus. Quantitative studies have been made of the main indole derivative of tryptophan, 5-hydroxyindoleacetic acid and of the following derivatives of the kynurenine pathway kynurenine, anthranilic acid, 3-hydroxyanthranilic acid and xanthurenic acid

Material and methods

The material comprised 10 diabetics — 4 females and 6 males. Their ages varied from 10 to 68 years (average 41.8) Seven of the cases were treated with insulin and 3 by dietetic means only The control group consisted of 12 hospitalized subjects with no known disease. Their ages varied from 19 to 61 years (average 32.8)

All medication except insulin was discontinued one day before the experiment. The determination of tryptophan metabolites was

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Table II Urinary excretion of tryptophan metabolites by patients with diabetes mellitus and healthy controls

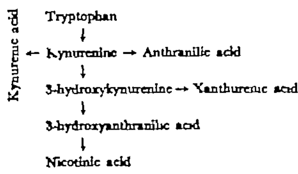
	No. of cases	Before (B) or after (A) tryptophan	Urinary tryptophan metabolites (µM/24 hrs)				
			5-hydroxy-indoleacetic acid	Kynurenine	Anthranilic acid	3-hydroxy-anthranilic acid	Xanthurenic acid
Diabetes mellitus	10	B	59.5 16–60	9.8 5–16	6.9 4–14	44.7 29–60	72.4 34–141
		A	53.1 23–95	15.2 7–27	9.7 3–18	64.3 49–86	128.9 58–263
Controls	12	B	28.8 12–57	14.7 7–31	10.4 1–21	57.1 11–82	14.8 4–26
		A	33.0 24–47	39.3 11–89	13.3 3–24	56.5 27–134	28.4 10–65

erythematosus, pregnancy (Price 1958) rheumatoid arthritis, tuberculosis, renal diseases and cancer of varying sites (6) Tryptophan metabolism may also be altered by drugs with antipyridoxine activity (Price) The metabolic patterns found (quantitative or paper chromatographic) have not proved specific for any condition except the carcinoid syndrome, in which large quantities of 5-hydroxyindoleacetic acid and other indole derivatives are excreted, and certain rare congenital errors of metabolism (Hartnup disease, phenylketonuria) The same holds true for the various metabolites of tryptophan. No really abnormal or specific metabolites have yet been demonstrated in any disease.

The previous studies on tryptophan metabolism in diabetes mellitus have indicated an abnormality (6, 7 10) However the results obtained by Rosen et al. and on the other hand by Kalant et al. were quite different. Rosen group studied only the 24-hour excretion of xanthurenic acid, which appeared to be increased. Kalant et al. measured the urinary excretion of tryptophan, xanthurenic acid, kynurenine, anthranilic acid and 5-hydroxyindoleacetic acid. They used a 2-hour basal urine specimen and after oral administration of tryptophan a 6-hour specimen. This might be the reason for the differing results. We feel that 24-hour urine collection is essential in diabetics, in whom the rate of urine excretion varies widely

Our studies confirm the results of Rosen et al. We also found an increased xanthurenic acid excretion in diabetics. Both basal xanthurenic acid excretion and that after the loading dose of L-tryptophan were markedly elevated This was the most noteworthy abnormality in the excretory pattern of tryptophan metabolites observed in our study In addition, the excretion of kynurenine was diminished compared to normal and the 5-hydroxyindoleacetic acid excretion slightly enhanced after tryptophan loading

Table I An abbreviated scheme of the metabolism of tryptophan by the kynurenine pathway



performed on two consecutive days from 24-hour urine. The first test day was a control day. On the morning of the second day a dose of 2 g of L-tryptophan (9.8 mM) was administered. The collection of urine was controlled by creatinine estimations and the possible forbidden use of interfering drugs by p-aminobenzoic acid determinations. Toluene was used as preservative.

Creatinine excretion varied in the diabetics from 0.37 to 1.60 g/24 hours (average 0.85) and in the controls from 0.69 to 1.75 g/24 hours (average 1.12). Urinary excretion of p-aminobenzoic acid ranged in the diabetics from 4 to 14 μM/24 hours (average 7.5) before tryptophan and from 5 to 37 μM/24 hours (average 13.2) after the tryptophan load. The corresponding figures in the controls were as follows: before tryptophan 7—31 (average 12.4) and after the tryptophan load 9—49 (average 19.0).

5-hydroxyindoleacetic acid was estimated by the method of Hanson and Serin (1), kynurenine, anthranilic acid, p-aminobenzoic acid and 3-hydroxyanthranilic acid according to the methods of Tompsett (9) and xanthurenic acid by the method of Rosen et al. (8).

Results

A derangement of tryptophan metabolism in diabetes mellitus was observed in this study. The most impressive abnormality was the markedly increased urinary excretion of xanthurenic acid both before and after the administration of the loading dose of L-tryptophan. In the diabetics the basal xanthurenic acid excretion exceeded the normal range in all of the cases and the mean excretion was about five times higher than in the control group. The ratio did not alter after tryptophan loading when 8 cases out of 10 presented values above the upper limit of the normal range.

Another abnormality of tryptophan metabolism found in the diabetics was the lowered urinary excretion of kynurenine after tryptophan loading, during which the excretion of kynurenine was on average 2.6 times lower than in the controls. (The difference is statistically significant, u = 2.92 in the Wilcoxon test, $P < 0.01$.)

The urinary excretion of anthranilic acid and 3-hydroxyanthranilic acid in diabetes mellitus did not differ significantly from that of the controls.

The urinary excretion of 5-hydroxyindoleacetic acid seemed to be slightly higher in the diabetics than in the control group. Two of the cases presented abnormally high values before trytophan loading and 6 cases after it. (The difference is statistically significant after the tryptophan load u = 3.00 $P < 0.01$.) However, no markedly elevated individual values were recorded (tables I and II).

There appeared to be no difference in the urinary excretory pattern of tryptophan metabolites between the diabetics receiving insulin and those treated by dietetic means alone.

Discussion

Disorders of tryptophan metabolism are not uncommon. Abnormal metabolic patterns have been found in several conditions including bladder cancer, porphyria, scleroderma, disseminated lupus

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From the Medical Department A, Rigshospitalet (Head K. Brøchner Mortensen, M D)
Copenhagen, Denmark

The Effect of a Single Dose of a Long-acting Anabolic Steroid (Anadur) in Patients with Osteoporosis

By

Uffe Sagild

In the attempt to develop steroid hormones with prolonged effect, esters of p-alkoxyphenyl propionic acid were recently introduced (2) By varying the length of the alkoxy group between 3 and 12 carbon atoms, long-acting esters of testosterone 19-nortestosterone, 17 beta-oestradiol, 17-alpha-hydroxy-progesterone, 11-deoxycorticosterone and cortisone were obtained

One of these esters, 19-nortestosterone p-hexoxyphenyl-propionate (*Anadur*) showed in animal experiments prolonged and powerful anabolic properties combined with a favourable anabolic/androgenic ratio These facts seemed to justify a clinical trial.

Four patients with varying degrees of osteoporosis were studied

Case reports

Case 1 A 72-year-old woman with osteoporosis of the spine of about two years duration. Spontaneous fractures of 6th, 9th and 11th thoracic vertebrae Regimen ambulatory Formula diet (see below) Control period of fifteen days, following which the nitrogen and calcium balances were observed for further 10 days after single intramuscular injection of 150 mg of *Anadur*[1] On the fifth day of the control period 53 microcuries of ¹³¹-I-labelled albumin was injected intravenously and the radioactivity of the plasma was followed daily through the remaining part of the study

Case 2 A 72-year-old woman with osteoporosis of the spine of about one year duration. Spontaneous fracture of the first lumbar vertebra. Regimen ambulatory Formula diet. Control period of 8 days, following which the balances of nitrogen and calcium were observed for further 12 days after a single intramuscular injection of 150 mg of *Anadur*

Case 3 A 45-year-old man with osteoporosis of the spine and low-back pain of about two months duration. No fractures. Regimen ambulatory Formula diet. Control period of 5 days, then the balances of nitrogen and calcium were followed for a further 21 days after single intramuscular injection of 50 mg of *Anadur*

Case 4 A 60-year-old man with slight osteoporosis of the spine and musculature atrophy probably secondary to familial episodic adynamia. Regimen ambulatory Formula diet, which due to lack of appetite contained only 7.9 g of nitrogen/24 hours. Control period of 5 days, then the nitrogen balance was followed for further 21 days after a single intramuscular injection of 50 mg of *Anadur*

[1] Kindly supplied by A/B LEO Hälsingborg, Sweden.

Submitted for publication August 29, 1962.

It is generally accepted that an increased urinary excretion of xanthurenic acid is a sign of pyridoxine deficiency. Vitamin B_6 in the form of pyridoxal phosphate is required for the metabolic breakdown of tryptophan at several metabolic steps (as the coenzyme of both kynureninase and 5-hydroxytryptophan decarboxylase). A deficiency of this coenzyme is followed by increased formation of quinoline compounds — xanthurenic and kynurenic acids. Thus the derangement of tryptophan metabolism observed in our study might be connected with a deficiency of vitamin B_6 in diabetes mellitus. The possibility that vitamin B_6 deficiency and consequently disordered tryptophan metabolism might contribute to the development of certain diabetic complications deserves further study.

It should also been borne in mind that tryptophan has been shown to be involved in the regulation of blood sugar as the precursor of a competitive inhibitor of insulinase (4) and that hyperglycemia can be induced in rabbits by 5-hydroxytryptophan which is the precursor of 5-hydroxytryptamine (2).

Summary

The metabolism of tryptophan was studied in 10 patients with diabetes mellitus and 12 control subjects by determining the urinary excretion of 5-hydroxyindoleacetic acid kynurenine anthranilic acid 3-hydroxyanthranilic acid and xanthurenic acid before and after a loading dose of 2 g of L-tryptophan.

A disorder of tryptophan metabolism was observed. This appeared as a markedly increased excretion of xanthurenic acid both before and after the loading dose of L-tryptophan. The excretion of kynurenine was diminished compared to normal and that of 5-hydroxyindoleacetic acid slightly enhanced after the tryptophan load.

It is suggested that the metabolic derangement observed is at least partially due to a deficiency of vitamin B_6.

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Fig. 3. Plasma disappearance curve for ¹³¹I-labelled albumin (case 1)

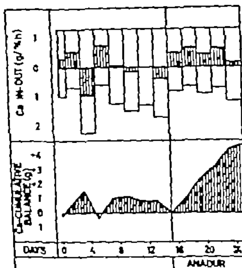


Fig. 4. The effect of Anadur on calcium balance (case 1).

least two weeks in those subjects responding.

Although the balances were not studied beyond this period the form of the curves suggest that nitrogen retention was maintained for a considerably longer period.

Fig 3 represents the plasma disappearance curve of ¹³¹I labelled albumin in patient No 1 It is seen that following an initial phase of five days during which the plasma activity declines faster the degradation of labelled albumin is exponential with time and is unaffected by the administration of *Anadur* (4)

2. *Calcium balance*

Fig 4 shows the effect upon the calcium balance of a single injection of *Anadur* (150 mg) in patient No. 1 It is clearly seen that the injection induced a strongly positive calcium balance in this patient. Similar results were obtained in patients Nos. 2 and 3 in whom the calcium balance was studied

3. *Side effects*

One patient (No. 2) developed muscular restlessness, nervousness, tachycardia and a temperature of 39 C two hours after the injection of 150 mg of *Anadur* She received 25 mg of chlorpromazine by mouth and 25 mg intramuscularly 30 min. later The subjective sensations wore off a few hours later but the temperature remained elevated during the next two days and a slight rise of GO-transaminase and GP-transaminase was noted two days after the injection. This elevation of the transaminases was transient and disappeared gradually during the following 10 days. The anabolic effect of *Anadur* as measured by nitrogen and calcium retention, continued beyond this period. The thymol turbidity and the concentrations of serum bilirubin, serum calcium and serum creatinine remained normal, and no albuminuria appeared.

None of the three other subjects experienced any immediate side effects of the injection. The GO- and GP transaminases, serum bilirubin, thymol turbidity and serum creatinine (checked twice weekly throughout the study) remained normal.

No virilizing or progestational effects were noted in any of the subjects.

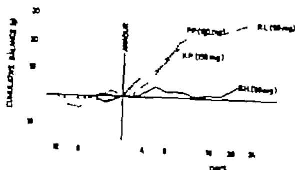


Fig 1 Cumulative nitrogen balance for 4 patients with osteoporosis before and after a single injection of Anadur

Methods

The metabolic technique has been described elsewhere (1) During the entire period of the study all patients received a formula diet, consisting of milk powder corn-flakes and biscuits, purchased in bulk for each investigation. The amount of each ingredient in the diet was decided upon in each individual case after a three-day trial period before the study Subsequently the diet was maintained constant throughout the study

Analyses of diet, urine and stools for nitrogen and calcium were carried out at the metabolic laboratory The nitrogen analyses were performed by Kjeldahl's method. Diet and feces were analyzed for calcium by Cramer and Tisdal's method in Hastrup's modification (3) urine by Sobel and Hauch's method (5)

Terminology Th terms "in-out balance" and cumulative balance are used in the senses defined previously in papers from this department (1)

Results

I Nitrogen balance

Fig 1 shows the cumulative balances of nitrogen in the four subjects following administration of 150 150 50 and 50 mg of *Anadur* respectively It is clearly seen that while three of the subjects (Nos 1 2 and 3) responded with a definite positive nitrogen balance, the fourth hardly responded at all. The explanation of the latter finding is not clear It may

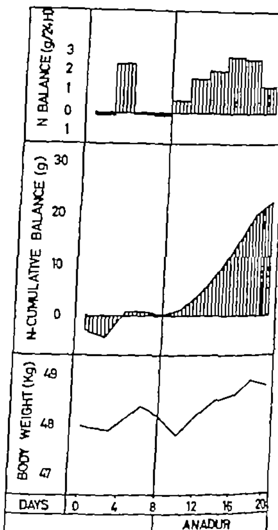


Fig. 2. The effect of Anadur on nitrogen balance (case 2)

be related to the poor protein intake throughout the study which was a consequence of the patient's lack of appetite, or it may be caused by inherent insensitivity to the drug due to his metabolic disease (familial episodic adynamia)

It is of interest to note that the anabolic response in patient No 3 who received 50 mg of *Anadur* is of the same order of magnitude as that found in patients Nos. 1 and 2 each of whom received 150 mg

Under the conditions of the study a single injection appears to have maintained a powerful anabolic effect for at

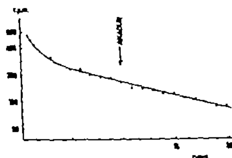


Fig. 3. Plasma disappearance curve for ¹³¹ I-labelled albumin (case 1)

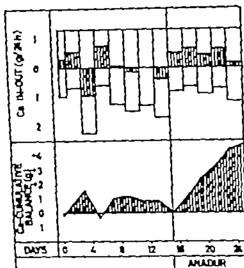


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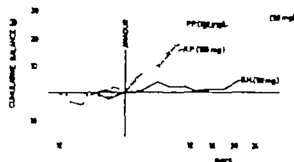


Fig 1 Cumulative nitrogen balance for 4 patients with osteoporosis before and after a single injection of Anadur

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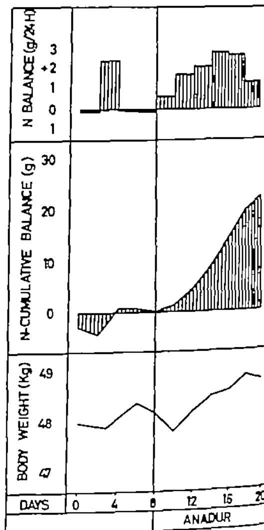


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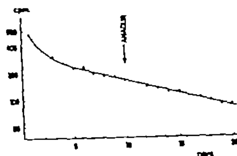


Fig. 3. Plasma disappearance curve for ¹³¹ I-labelled albumin (case 1).

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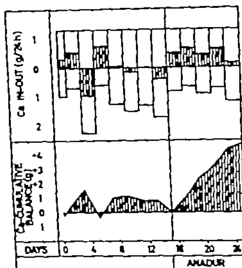


Fig. 4. The effect of Anadur on calcium balance (case 1).

Two of the patients (*Nos. 1 and 4*) have been studied two and six months after the injection, respectively There were no signs of late side effects at this time and the liver function tests were normal.

Anadur has also been subjected to clinical trials in Sweden and according to personal communications from Ahwall et al no side effects were observed in a group of 45 patients having received a total of 245 injections

Summary

A long-acting anabolic steroid (*Anadur*) has been tested in balance studies in four patients with osteoporosis. One of these had in addition muscular atrophy probably secondary to familial episodic adynamia.

A definite anabolic effect, as shown by retention of nitrogen and calcium was observed in two patients receiving a single dose of 150 mg intramuscularly and in one receiving 50 mg The fourth patient (with familial episodic adynamia) did not respond to a dose of 50 mg

The balance data suggest that the single injection maintained a powerful anabolic effect for a period of two weeks, and the form of the curves suggests a considerably longer duration in patients responding

The rate of degradation of intravenously administered ^{131}I labelled albumin was not affected by the anabolic steroid in one patient studied

An undesirable side reaction, in some way related to Anadur and possibly also to the subsequent administration of another drug (chlorpromazin) was observed in one patient.

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A Histological Investigation of Kidney Biopsies in Cushing's Syndrome

By

P. M. Arkenbout, J. de Graeff and A. J. te Rijdt

During the surgical treatment of 19 patients with Cushing's syndrome, one or more biopsies were taken from one or both kidneys for histological investigation. Hypertension is seldom absent from the symptomatology of this syndrome and frequently determines the prognosis of the disease. It therefore seemed important to find out whether there are histological abnormalities in the kidneys which are secondary to this hypertension, and whether after adequate treatment of Cushing's syndrome the further course of the hypertension is determined by the severity of this histological abnormality. This possibility is supported by results of the present investigation.

In addition to vascular abnormalities, other histological changes were also found, namely calcium deposits in the tubuli and pyelonephritic changes. Histological signs of potassium depletion were not observed.

Submitted for publication September 3, 1962.

Clinical data and methods

The most important data from the patients are given in tables I and II. In 18 of the 19 cases a hyperplasia of the adrenal gland was found, while in 1 patient (No. 17) an adenoma of the adrenal cortex was responsible for the Cushing's syndrome. Of the 19 cases, 15 were women and 4 men. 12 fell in the age group 20 to 40 years. In all cases the diagnosis of Cushing's syndrome was based on the clinical picture and the results of the laboratory examination.

In 12 patients therapy consisted exclusively of unilateral adrenalectomy followed by irradiation of the pituitary. In 6 other patients this procedure did not give adequate results and a subtotal or total adrenalectomy was subsequently done on the other side. These 6 patients all required a substitution dose of cortisone, and a few also received DOCA. In 1 patient (No. 17) one adrenal gland with a cortical adenoma was removed. In 17 of the 19 patients complete clinical remission was obtained. One patient (No. 16) refused further therapy after a unilateral adrenalectomy and irradiation of the pituitary even though complete recovery had not yet been achieved. One

Table I Clinical data

	Age (yrs)	Sex	Duration of symptoms (yrs)	B. P (mm Hg)	Operation	Irradiation pituitary
1	22	♀	3	135/105—120/90	1953 L	+
2	36	♀	10	160/120—130/90	1957 R	+
3	27	♀	0.5	160/110	1954 R	+
	28		1.5	160/105	*1955 L	
4	16	♀	1	190/115	1951 R	+
	21		6	145/105—140/85	1956 L	
	23		8	120/90	1958 L	
5	15	♂	3	120/70	1956 R	
6	22	♀	0.5	150/120—130/90	1953 R	+
	23		1.5		1954 L	
	26		4.5	130/85 —160/100	1957 L	
7	53	♀	15	170/110—145/95	1953 L	+
8	24	♀	4	130/110—120/85	1953 L	+
9	28	♂	3	150/120—115/85	1957 L	+
10	43	♀	2	225/145—160/100	1956 L	+
11	51	♀	4	160/110	1956 R	+
12	30	♀	4	170/115—145/100	1958 R	+
13	31	♀	1	160/110	1956 R	+
14	41	♀	8	220/150	1955 R	+
	42		9	180/130	1956 L	
15	21	♂	3	210/140	1947 L	+
	27		9	170/140	1953 R	
16	32	♀	7	150/110	1954 L	+
17	47	♀	8	160/100	1957 R	
18	56	♀	11	180/100	1957 R	+
19	30	o	2	200/150	1956 L	+
	31		3	200/150	1957 R	

L = left adrenal emoved R = right drenal removed.

renal calculus subtotal adenoma.

patient (No. 14) showed, in addition to a bilateral adrenal hyperplasia, radiological symptoms of a tumor of the hypophysis. This patient also refused further treatment. A bilateral adrenalectomy had not produced complete remission of the clinical symptoms of Cushing's syndrome.

In 19 patients a biopsy sample was taken from one of the kidneys. In 4 of these a biopsy was done on the other kidney during a subsequent operation, making a total of 23 biopsies. The tissue samples varied in size, the average dimension being 8×5×4 mm. They were fixed immediately in 4 % formalin and embedded in paraffin. The sections were cut 5 μ thick. The following stains were used hematoxylin and eosin Weigert's elastic stain, van Gieson's connective tissue stain, van Kossa's stain for calcium, periodic acid Schiff stain (PAS) and PAS colloidal iron.

The histological changes were divided according to severity into four classes, 0—IV. The severity of the hypertension was also divided into four classes, 0—IV on the basis of the clinical picture.

a. Classification of the histological abnormalities

It is obvious that a biopsy sample which contains only a small portion of the kidney and even then only of the cortex, cannot provide material for extensive conclusions about the presence of abnormalities in the kidney

tissue as whole. Particularly the presence of pyelonephritis might be missed. Diffuse abnormalities, however will be apparent. On the basis of Smith's (10) investigations of changes in the kidney accompanying essential hypertension, it may be assumed that arteriolosclerotic changes are diffusely present throughout the entire kidney and that a kidney biopsy will provide some decisive evidence.

It is essential that the histological changes in the kidneys be graded. In the literature various classifications are used (2, 8) On the basis of the experiences of Heptinstall (4) and Saltz w have given preference to division into classes 0—IV in which special attention is paid to the hyaline changes in the arterioles, although the glomeruli and tubuli are also included in the investigation.

Heptinstall (4) and Saltz (8) found certain correlation between the abnormalities in the arterioles, the degree of hyalinization of the glomeruli, and the atrophy of the tubuli. The presence of extensive round-cell infiltrations in the cortex without hyalinization of the glomeruli, and of focal interstitial fibrosis with atrophy of tubuli containing eosinophilic cylinders without diffuse abnormalities of the arterioles, were taken as indications of chronic pyelonephritis.

Limited abnormalities are found in groups I and II and more extensive abnormalities in groups III and IV In number of cases signs of chronic pyelonephritis (P) were found. These biopsies, in which the atrophy of tubuli was sometimes disproportionately extensive in relation to the changes in the arterioles, were classified according to the severity of the remaining abnormalities (e.g III P) None of the biopsies showed such extensive changes as to require placement in group IV

b Clinical classification according to the severity of the hypertension

As the literature indicates, it is extremely difficult to classify the clinical severity of the hypertension. I order to be able to look for relation between histological changes in the kidneys and the severity of the hypertension, however such classification was indispensable, and we arbitrarily chose that of Schroeder (9) In this classification the criteria for hypertension are the height of the

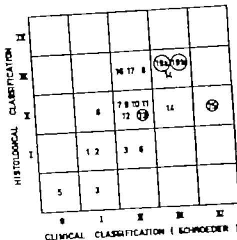


Fig. 1. Relationship between the severity of the histological abnormalities in the biopsies and the clinical severity of the hypertension.

diastolic pressure and changes in the eye grounds and kidney function, no attention being given to cardiac and cerebral complications deriving from the hypertension.

Results

A. CHANGES IN THE ARTERIOLES OF THE KIDNEYS

Fig 1 shows schematically the relationship between the severity of the histological abnormalities in the kidneys and the clinical severity of the hypertension. It is clear that there is a good correlation between these two sets of data. For 13 of the 23 biopsies the classification was the same according to both criteria. The clinical severity of the hypertension was classified differently from the histological abnormalities 9 times 5 times in one class lower and 4 times in one class higher Only in one patient (No. 15) were few (primarily pyelonephritic) abnormalities found in the biopsy sample (class II) while the hypertension was clinically severe (class IVa)

Table II Clinical data

	Age (yrs)	B. P (mm Hg)	Heart size	ECG	Eye grounds	Urine	
						Prot.	Sed.
1	22	135/105—120/90	0	0	0	0	0
2	36	160/120—130/90	0	L. S.	0	Tr	0
3	27	160/110	0	0	0	0	0
	[1]28	160/105			II	+	WBC
4	16	190/115				0	0
	21	145/105—140/85				0	0
	23	120/90				0	0
5	15	120/70	0	0	0	0	0
6	22	150/120—135/90				Tr	10—15 WB
	23						
	26	130/ 85—160/100	0	L. S.	I	Tr	2—4 WBC
7	33	170/110—145/95	0	L. S.	0	0	0
8	24	130/110—120/85	0			0	0
9	28	150/120—115/85	L. H.	L. S.	I	Tr	0
10	43	225/145—160/100	0	0	II	0	0
11	51	160/110	L. H.	L. S.	II	+	2—4 WBC
12	30	170/115—145/100	0	0	0	Tr.	0
13	31	160/110	0	0	0	Tr	2—4 WBC
14	41	220/150	L. H	L. S.	II	Tr.	10—15 WBC
	42	180/130	L. H.	L. S.	II	Tr	10—15 WBC
15	21	210/140	L. H.		I	Tr.	Some cyl.
	27	170/140		L. S.	IV	Tr	0
16	32	150/110	0	0	0	Tr	15—20 WBC
17	47	160/100	L. H.	L. S.	II	Tr	2—5 WBC
18	56	180/100	L. H.	L. S.	II	Tr	10—15 WBC
19	30	200/150	L. H.	0	I	0	3—5 RBC
	31	200/150		0	II	T	0

L. H. = hypertrophy left ventricle L. S. = left strain T = trace of protein WBC = white blood excretion of intravenously injected phenolsulphonephtalein 15 and 60 min after injection. If only one in percent of normal P = inflammatory changes N = normal D = diabetic. Renal calculus

In all patients whose kidney biopsy was assigned to classes 0—I after treatment (table II) the blood pressure dropped to normal or almost normal. Of the 8 patients whose kidney biopsy belonged to class II after adequate treatment the blood pressure was normal in 2 cases, slightly elevated (class I) in 2 cases, greatly improved (from class IVa to class II) in 1 case and unchanged in 3 cases (class II) Of the 3 patients whose biopsies belonged to class III after otherwise adequate treatment the blood pressure was still slightly elevated (class I) in 1 case (adenoma removed) somewhat improved in 1 case (from class III to class II) and unchanged in 1 case (class II) In these last two biopsies, signs of pyelonephritis were found

Two patients, as indicated in the introduction were not adequately treated (Nos 14 and 16) Neither was a case of

Renal function			Glucose tolerance test	B.P after surgery	Classification		
					Clinical[?]		Histological
S.G.	P.S.P (%)	U.Cl (%)			Before	After	
1,022	37/23		N	125/85	I	0	I
	35/27		D	130/80	I	0	I
1,025			D	140/100	II	I	I
1,021		95		120/90	I	I	0
1,020	47/58		N	140/105	II	I	
1,023	48 34			120/90	I	0	I
	24/20			120/90	0	0	
	41/35	144	N	120/70	0	0	0
				125/90	II	I	I
				125/95	I	I	
	29/31	76	N	120/90 —155/100	I	I	II
	73		N	150/100—140/95	II	I	II
1,028	14/28		N	120/80	I	0	II
	60 30		D	135/80	II	0	II
	31/33	71	N	180/115—160/100	II	II	II
	38/24	68	D	170/120	II	II	II
1,025	34/30		D	155/90	II	I	II
	29/30		N	160/100	II	II	II P
	29/16	46	D	180/130	III	III	II
	37/23	79		190/150	III	III	III
	33		N	200/150	III	III	
1,027	29/25	78		155/110	IV	II	II P
	32 34		N	140/95	II	I	III
	34/18	64	D	130/90	II	I	III
		45	D	180/115	II	II	III P
	27/28	71	N	200/130	III	III	III P
1,020		115		170/110	III	II	III P

cells per high power field; S.G. = specific gravity of the urine after fluid restriction P.S.P = number is given this indicates the excretion of the dye 2 hrs after injection U Cl. = urea clearance According to Keith-Wagener; According to Schroeder; According to Heptinstall and Saltz.

clinical remission. They were excluded from the evaluation of the effect of adequate treatment on the hypertension.

On the whole, it can thus be said that adequate treatment led to improvement in the status of the hypertension. The degree of the *histological* abnormalities in the kidneys gave some grounds for the prognosis of the hypertension after surgery The term "adequate treatment" will be discussed further below

B. OTHER HISTOLOGICAL ABNORMALITIES

Calcium deposits

In 17 of the 23 biopsies, *minor* calcium deposits were found in the tubuli. This applies, of course, only to the cortex. Since the clinical *examination of the* patients did not include calcium metabolism, the data on this point are incomplete. The calcium content of the blood was determined in 17 of the 19 patients. It in fact fell consistently within normal

Table II Clinical data

	Age (yrs)	B. P (mm Hg)	Heart size	ECG	Eye grounds	Urine	
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8	24	130/110—120/85	0			0	0
9	28	150/120—115/85	L. H.	L. S.	I	Tr	0
10	43	225/145—160/100	0	0	II	0	0
11	51	160/110	L. H.	L. S.	II	+	2—4 WBC
12	30	170/115—145/100	0	0	0	Tr.	0
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from it. It seemed worth-while to investigate how far the histological picture of the kidney reflects the clinical picture. The findings indicated that the histological picture is primarily determined by the changes which are to be expected with hypertension and there is no effect from the other manifestations of the disease.

The correlation between the severity of the histological abnormalities in the kidneys and the clinical severity of the hypertension has been investigated, by among others, Saltz (7). He used Smithwick's classification (6 classes) to define the pre operative condition of the patient. This classification takes account not only of the blood pressure but also of other clinical data such as age, condition of the brain, heart, kidneys, fundus of the eye, and the reaction of the blood pressure to sedatives. There appeared to be a reasonable degree of correlation between the histological and clinical data.

Heptinstall (4) compared the pre operative diastolic pressure with the histological abnormalities in the kidneys. He too found some although not complete, correlation between both data. For our patients with Cushing's syndrome a similar rather good correlation was also found. As criteria such as the severity of the changes in the eye grounds play an important role in the clinical classification of the severity of the hypertension, such a correlation is perhaps not too surprising some relationship between these vascular changes in the eye grounds and those in the kidney can be expected.

No correlation was found between the presence of diabetes mellitus and the severity of the histological changes in the kidneys. The severity of the other symptoms (atrophy of the skin, osteoporosis, excretion of neutral 17 ketosteroids, etc.) also failed to provide any grounds for prediction concerning the kidney biopsy.

It is sometimes difficult to determine whether a given therapy has been adequate for patients suffering from Cushing's syndrome. In some cases the patient's appearance becomes entirely normal, the hematological abnormalities disappear while the hypertension remains and for example the excretion of corticosteroids is still above normal. We considered the therapy adequate when both the external symptoms had disappeared and laboratory and other examinations were unable to demonstrate any further signs of activity. With this reservation, it may be said that in most of the cases adequate treatment produced an improvement in the clinical symptoms of the hypertension. The kidney biopsy here permits the drawing of a few conclusions about the prognosis of the hypertension. After surgery the blood pressure became normal when the kidney biopsy had been assigned the classes 0—I. In the histological class II the post-operative blood pressure remained slightly elevated in half of the cases. In two of the three cases in histological class III the blood pressure remained distinctly high. In these cases histological signs of inflammation were also found.

The incompleteness of the clinical data makes it difficult to form an opinion about the importance of the occurrence of focal inflammation infiltrates. In fact, too little is known in general concerning the relationship between the histological signs of inflammation and the occurrence of bacteriologically demonstrable infections.

The frequency of minor calcium deposits in the tubuli is remarkable. The investigation of the calcium metabolism was not sufficiently complete to permit a con-

limits at least if no importance is attached to an occasional value between 11 0 and 11 6 mg % in patients with otherwise normal values.

Calcium excretion in the urine during a low calcium diet was determined in only 6 patients There were found in one patient (No 4) a slightly increased value of 230 mg/24 hours, and in another patient (No. 7) one normal value (109 mg) and one slightly increased value of 235 mg/24 hours. In these two patients only a slight amount of calcium deposit was found in the biopsy The other values all fell within normal limits. Two patients had kidney stones. Both excreted normal quantities of calcium in the urine.

No correlation could thus be found between the severity of the calcium deposits in the kidneys and the abnormalities in calcium metabolism.

Signs of potassium depletion

None of the biopsies showed signs of potassium depletion such as vacuolar degeneration in the cells of the proximal tubules. In 18 of the 19 patients the potassium content of the blood was determined one or more times. It was consistently normal except in patient No 14 in whom we found once a value of 3 2 mEq/l and once a value of 4 0 mEq/l In patient No. 19 the K content of the serum was 3 4 mEq/l but this determination was made only once.

Thus clinical indications of potassium depletion in this group of patients were not found.

Signs of pyelonephritis

In 4 patients (Nos. 13 15 18 and 19) the biopsies showed signs of chronic inflammation. In 2 of them no suggestion of an infection of the urinary system could be found either from the anamnesis or in the sediment. One patient (No. 19) had a stone in the right ureter but no signs of inflammation were found in the sediment. The urines of these patients were not cultured bacteriologically so that no definite conclusions may be drawn concerning the presence of an infection of the urinary system.

In two other patients (Nos. 3 and 14) an infection of the urinary system was found even though there were no histological signs of inflammation in the kidney biopsy One of the patients had a nephrolithiasis (No 3) Histological signs of inflammation may however be absent from the biopsy sample because of their focal localizations.

Other abnormalities

None of the abnormalities which were suggested by Kark (5) to be characteristic for Cushing's syndrome were found.

Discussion

Little is known concerning the histological picture of the kidneys of patients suffering from Cushing's syndrome. Kark (5) suggested that the picture might show specific abnormalities. He described two cases in which all the glomeruli showed a dilation of the glomerular capillaries which resembled the capillary swelling seen with eclampsia. The distended region did not take PAS stain and disappeared after the operation. We were not able to confirm this in our material.

Cushing's syndrome shows a varied clinical picture. In some patients the hyperglycemia predominates, in others the signs of catabolism are the most striking In most patients, however the clinical picture is dominated by the hypertension and the complications which derive

from it. It seemed worth-while to investigate how far the histological picture of the kidney reflects the clinical picture. The findings indicated that the histological picture is primarily determined by the changes which are to be expected with hypertension and there is no effect from the other manifestations of the disease.

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The frequency of minor calcium deposits in the tubuli is remarkable. The investigation of the calcium metabolism was not sufficiently complete to permit a con-

4-5.

clusive opinion. It is assumed that the decalcification of the skeleton in Cushing's syndrome is due to a decreased amount of bone matrix. The calcium content of the blood shows no increase. It is conceivable that during the onset of the decalcification a period of increased calcium excretion results in these calcium deposits

The absence of vacuolar degeneration in the cells of the proximal part of the tubulus agrees with the absence of clinical signs of potassium depletion.

Summary

During surgical treatment of nineteen patients with Cushing's syndrome, one or more biopsy samples were taken from the kidneys. These samples were investigated histologically No changes specific for Cushing's syndrome were found Fair correlation was found between the clinical severity of the hypertension and the histological picture. In most of the cases, adequate treatment brought about a reduction of the hypertension. The prognosis can to a certain extent be inferred from the severity of the histological abnormalities. In addition to vascular changes, a few of the biopsies showed signs of pyelonephritis. In most of the biopsies calcium deposits in the tubuli were found

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The Defibrination Syndrome in a Patient with Haemangio-endothelio-sarcoma

By

Sverre Blix and Carl Ditlef Jacobsen

The theory of continuous intravascular coagulation as a physiological process has not been proved. However, there is reason to believe that such a process may occur in various pathological states, resulting in specific coagulation changes, hypofibrinogenaemia and thrombocytopenia (the defibrination syndrome). The condition may be accompanied by a severe bleeding tendency.

The purpose of this work is to present further evidence for the existence of this syndrome and to obtain information about the underlying mechanism.

The history reported is of a patient with a malignant intramuscular haemangioma in the left hip region, and severe local bleeding. The laboratory findings strongly suggested a defibrination syndrome. The result of anticoagulant treatment supported this view. Unfortunately the patient died after a complicating intrapleural haemorrhage.

Submitted for publication August 27, 1962.

Case report

A 68-year-old farmer. There was no history of haemorrhagic disorders in his family. He had been in good health, except for bilateral dysplasia coxae with secondary arthrosis. In 1923 he had trauma in the region of the left hip. Pain and stiffness started in 1930, chiefly in the left hip joint. He received X-ray treatment in 1942. Bleeding tendency had never been recorded.

July 1961 in an accident, he fractured two metatarsal bones in the left foot and had a contusion against the truncus. A haematoma in the region of the left trochanter major disappeared within 5 weeks. At that time, as he started to walk on the left foot, a gradually increasing haematoma developed in the same place. On admission to the local hospital in Oct. 1961 he was found to be gravely anaemic (Hb 8.1 g/100 ml) with thrombocytopenia (60,000/mm^3) and his blood was nearly incoagulable. High doses of prednisone had no effect, and after blood transfusions the haematoma increased.

On Nov. 3rd, 1961 he was transferred to the University Hospital. He was still severely anaemic, slightly icteric, and his chief com-

clusive opinion. It is assumed that the decalcification of the skeleton in Cushing's syndrome is due to a decreased amount of bone matrix. The calcium content of the blood shows no increase. It is conceivable that during the onset of the decalcification a period of increased calcium excretion results in these calcium deposits.

The absence of vacuolar degeneration in the cells of the proximal part of the tubulus agrees with the absence of clinical signs of potassium depletion.

Summary

During surgical treatment of nineteen patients with Cushing's syndrome, one or more biopsy samples were taken from the kidneys. These samples were investigated histologically. No changes specific for Cushing's syndrome were found. Fair correlation was found between the clinical severity of the hypertension and the histological picture. In most of the cases, adequate treatment brought about a reduction of the hypertension. The prognosis can to a certain extent be inferred from the severity of the histological abnormalities. In addition to vascular changes, a few of the biopsies showed signs of pyelonephritis. In most of the biopsies calcium deposits in the tubuli were found.

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Table II Data on the haemostatic mechanism on admission

Examinations	Results	Normal range	Methods
Fibrinogen (mg %)	60	175—350	Jacobsson (13)
Platelet count (/mm)	38,000	140,000—350,000	Nygaard (15)
Primary bleeding time (min)	15—30	2—12	Borchgrevink & Waaler (6)
PP %	58	80—100	Owren & Aas (16)
Thromboplastin time (sec)	25.3	13—14	Quick system with human brain thromboplastin
Cephalin time (sec)	59.5	55—65	Egeberg (7)
Prothrombin (factor II) %	66	80—120	Hjort et al. (11)
Proaccelerin (factor V) %	40	80—120	Aas (1)
Proconvertin (factor VII) %	70	80—120	Owren & Aas (16)
Antihaemophilic A (AHA, factor VIII) %	68	60—150	Egeberg (7)
Antihaemophilic B (AHB, factor IX) %	100	70—140	Egeberg (7)
Antihaemophilic C (AHC, factor XI) %	92	70—140	Egeberg (7)
Platelet adhesiveness (%)	58	10—25	Hellem (9)
Fibrinolysis (sq. mm)	0	0—25	Standard plates, Astrup & Müllertz (5)
Fibrinolysis (plasma clot lysis time)	Not increased		Hjort (10)
Proactivator (%)	40	80—120	Blix (4)

Epsilon-amino-caproic acid was added to avoid fibrinolysis (4).

All coagulation determinations in this report were kindly carried out by Dr O Egeberg.

Estimated normal range t the patient's haematocrit value.

obtain haemostasis because of steady oozing of blood into the haematoma cavity after evacuation. On inspection, no local cause of the bleeding could be seen. There was no evidence of tumour or aneurism. The cavity slowly re-filled with blood, and two days after the operation the bleeding from the wound increased considerably and continued for eleven days. The small haematoma on the right side, however seemed to be in regression.

Besides the transfusions and surgical treatment, various drugs were tried without significant effect (prednisone diethylstilboestrole Adona and Premarin)

3 *Nov. 25th to Dec. 7th. Anticoagulant treatment*

At this time the cause of his fibrinogenopenia and thrombocytopenia was still obscure, but the possibility of continuous intravascular coagulation had been discussed several times. The patient's condition was now cachectic in spite of daily blood transfusions a fatal end seemed inevitable. However as last trial anticoagulant treatment with phenylindane dione was started on Nov 25th. W aimed t thrombotest level of about 10 %

Following this therapy a considerable rise in platelet count occurred, and transfused fibrinogen disappeared more slowly from the circulation than after earlier transfusions. The bleeding from the wound stopped.

After one week on treatment, however he developed signs of congestive heart failure, slight dyspnoea, increasing oedema and mucopurulent expectoration with streaks of blood *He* received digitalis, diuretics and

Table I Laboratory data on admission

Examinations	Results	Normal range
Haemoglobin (g %)	5	14—18
Erythrocytes (mill./mm³)	1 72	4.6—6.2
Osmotic fragility (beginning haemolysis) % N Cl	0.5	0.48—0.55
Haematocrit (%)	17	40—54
Leukocytes (mm³)	12,700	5.000—10.000
SR (mm)	2	2—10
Urea (mg %)	73	20—40
Creatinine (mg %)	1.2	0.5—2.0
Phosphatase (alk.) (Bodansky U)	1 7	2—5
Phosphatase (acid) (Bodansky U)	0	0—1
Calcium (mEq/l)	4.4	4 7—5.5
Phosphorus (inorg.) (mg %)	3.3	2.5—4.5
Total protein (g %)	6	6.2—7.5
Albumin (g %)	3.2	3.6—5
Coombs test	Negative	Negative
Cold agglutinins	Negative	Negative

plaints were weakness, and pain in the left hip and leg On the front and lateral part of the left hip a large haematoma could be seen, and on the opposite side in the same region a similar but smaller haematoma was found. This second haematoma had slowly appeared since mid-October Clinical examination revealed normal conditions, except for the arthrotic changes in the hip joints. No bleeding manifestations other than the haematomata could be found. Stools and urine showed negative reactions for blood The prostate was clinically normal. Because of his bad condition X-ray examinations were limited to the thorax and the hip joints. Except for the dysplasia coxae with arthrotic deformities there were no pathological findings.

Laboratory investigations

The laboratory data on admission is reported in tables I and II The urine was normal. Electrophoresis of serum was normal, except for a slight hypoalbuminaemia. Serological tests for lues, cultures for tubercl bacillus in the sputum, and blood cultures were all negative. Differential count of leukocytes in peripheral blood showed a normal distribution. Sternal marrow aspirated only once, soon after death, revealed an intense erythropoiesis with formation of multi-nucleated normoblasts. Megacaryocytes were present in normal amounts.

Course and treatment

The patient's bleeding was explained as a result of the fibrinogenopenia and thrombocytopenia. The variations in haemoglobin, platelets, fibrinogen, fibrinolysis and the most important clotting factors during his stay in the hospital from Nov 4th to Dec. 7th 1961 (death) are reported in figs 1 and 2 The trials of active therapy can conveniently be divided in three periods.

1 Nov 4th to 13th. Unsuccessful attempts at treatment by transfusions of blood fibrinogen and epsilon-amino-caproic acid

At the beginning the patient was treated by transfusions either of freshly collected blood or red blood cells. In spite of no detectable fibrinolysis at this time, we tried epsilon-amino-caproic acid (6 g every 6 hours for 48 hours) but the patient soon refused to take more of this drug An attempt was also made to correct his fibrinogenopenia with transfusions of fibrinogen derived from 3 l of fresh plasma (Cohn fraction I) or purified fibrinogen (Kabi ") 4 g Only an inadequate and shortlasting rise in the fibrinogen level without marked influence on the low platelet count was obtained. During the latter part of this period of multiple transfusions a varying fibrinolytic activity could be revealed on standard fibrin plates (fig 1)

2 Nov 14th to 24th. Unsuccessful result of surgical exploration of the haematoma

In spite of the intensive therapy the large haematoma constantly increased in size and a spontaneous perforation was feared. As a local cause of the bleeding could not be excluded, a surgical exploration was carried out on Nov 14th. Even with preoperative transfusions of blood, fibrinogen, epsilon-amino-caproic acid (see fig 1) and platelets separated from one litre of plasma, it was impossible to

Table II Data on the haemostatic mechanism on admission

Examinations	Results	Normal range	Methods
Fibrinogen (mg %)	60	173—350	Jacobsson (13)
Platelet count (/mm)	38,000	140,000—350,000	Nygaard (15)
Primary bleeding time (min)	15—30	2—12	Borchgrevink & Waaler (6)
PP %	56	80—100	Owren & Aas (16)
Thromboplastin time (sec)	25.3	13—14	Quick system with human brain thromboplastin
Cephalin time (sec)	59 5	55—63	Egeberg (7)
Prothrombin (factor II) %	66	80—120	Hjort et al. (11)
Proaccelerin (factor V) %	40	80—120	Aas (1)
Proconvertin (factor VII) %	70	80—120	Owren & Aas (16)
Antihaemophilic A (AHA, factor VIII) %	68	60—150	Egeberg (7)
Antihaemophilic B (AHB, factor IX) %	100	70—140	Egeberg (7)
Antihaemophilic C (AHC, factor XI) %	92	70—140	Egeberg (7)
Platelet adhesiveness (%)	58	10—25	Hellem (9)
Fibrinolysis (sq. mm)	0	0—25	Standard plates, Astrup & Müllertz (3)
Fibrinolysis (plasma clot lysis time)	Not increased		Hjort (10)
Proactivator (%)	40	80—120	Bliz (4)

Epsilon-amino-caproic acid was added to avoid fibrinolysis (4).

All coagulation determinations in this report were kindly carried out by Dr O. Egeberg.

Estimated normal range at the patient haematocrit value.

obtain haemostasis because of steady oozing of blood into the haematoma cavity after evacuation. On inspection, no local cause of the bleeding could be seen. There was no evidence of tumour or aneurysm The cavity slowly re-filled with blood, and two days after the operation the bleeding from the wound increased considerably and continued for eleven days. The small haematoma on the right side, however seemed to be in regression.

Besides the transfusions and surgical treatment, various drugs were tried without significant effect (prednisone diethylstilboestrole Adona and Premarin)

5. Nov. 25th to Dec 7th. Anticoagulant treatment

At this time, the cause of his fibrinogenopenia and thrombocytopenia was still obscure but the possibility of continuous intravascular coagulation had been discussed several times. The patient's condition was now cachectic; in spite of daily blood transfusions a fatal end seemed inevitable. However as last trial anticoagulant treatment with phenylindanedione was started on Nov 25th. We aimed at thrombotest level of about 10 %.

Following this therapy a considerable rise in platelet count occurred, and transfused fibrinogen disappeared more slowly from the circulation than after earlier transfusions. The bleeding from the wound stopped.

After one week on treatment, however he developed signs of congestive heart failure, slight dyspnoea, increasing oedema and mucopurulent expectoration with streaks of blood. H received digitalis, diuretics and

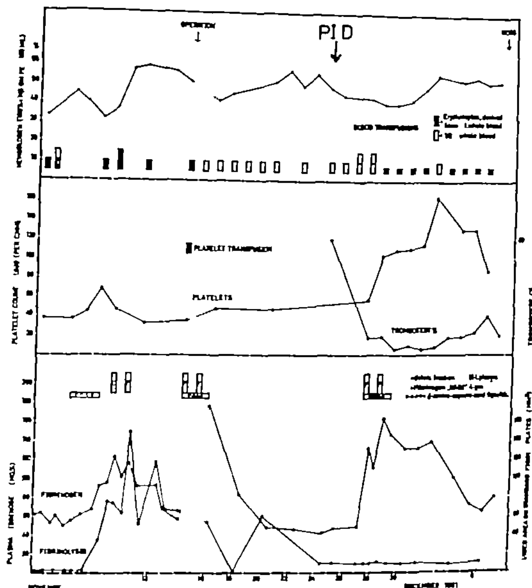


Fig. 1 The haemoglobin, platelets, TT %, fibrinogen and fibrinolytic activity during the patient's stay in the University Hospital. Anticoagulant treatment (phenylindanedione P I. D) was started on Nov 25th.

antibiotics. However thrombotest values had fallen below 5 % for a few days, and eight days after the anticoagulant therapy started, the bleeding from the wound again commenced. Now he became rapidly worse, the fibrinogen and platelet count again decreased, and he died in a cachectic condition on Dec. 7th. Autopsy examination revealed a large haemothorax, probably caused by the period of undesirable hypocoagulability when the thrombotest value fell below 5 %

Autopsy examination

There were 6 l of liquid blood in the right pleural cavity but a local cause of the bleeding was not found, and the lungs were normal. Macroscopically there were no signs of haemangioma or neoplastic growth in the region of the haematoma. Microscopically however the wall of the cavity showed diffuse infiltration with atypical cyncytial cells of epithelial-like appearance and lining spaces filled with blood cells (fig 3) By

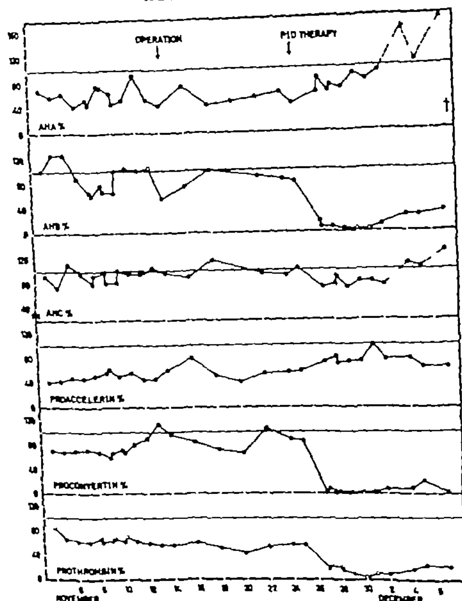


Fig. 2 The various clotting factors during the patient stay in the University Hospital.

specific staining no fibrin was detectable. I the left suprarenal gland small tumour (pea-size) with the same microscopical signs was found. The post mortem examination was otherwise without any important findings the liver spleen lymphatic glands and the prostate were normal, and the coronary arteries showed only slight atheromatous changes.

Histological diagnosis

Haemangio-endothelio-sarcoma in the lateral muscles of the left hip region. Small metastatic growth in the left suprarenal gland.

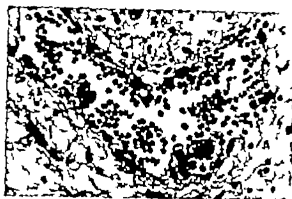


Fig 3 Section from the wall of the haematoma showing the haemangiomatous structure with partly detached atypical endothelial cells (Haematoxylin-eosin)

Discussion

The bleeding in this patient was probably due to the fibrinogenopenia and thrombocytopenia (on admission 60 mg % and 38,000/mm³) The pathogenesis could theoretically be explained in different ways.

I *Decreased production*

A. Theoretically a partial *congenital* deficiency of fibrinogen could not be ruled out but this possiblity seemed remote and would not explain the thrombocytopenia

B An *acquired* fibrinogenopenia caused by impaired liver function was improbable because there were no signs of liver disease

II *Increased consumption*

A. *Haemorrhage* Although the haematoma was large it could not account for a lasting fibrinogenopenia and thrombocytopenia.

B *Fibrinolysis* For the first four days no increased fibrinolytic activity could be revealed and cancer of the prostate was ruled out. A transitory fibrinolysis was recorded in connection with fibrinogen and blood transfusions. Further thrombocytopenia can hardly be exp by increased fibrinolytic activity

C. *Coagulation.* The hypothesi physiological and continuous intrava coagulation (2) has not been provec Many investigators, however acce existence of a *defibrination syndrome* has recently been reviewed by Go (8) The typical blood-changes i syndrome are reduced concentratic fibrinogen, proaccelerin antihaemo A factor and prothrombin and thrombocytopenia The same synd has been experimentally produce dogs by intravenous infusion of thro plastin (17 18)

By exclusion the most probable planation of the fibrinogenopenia thrombocytopenia in our patient w continuous coagulation, possibly gered by thromboplastic material f the haemangioma, with consequent t ping of platelets by the fibrin. Deta clotting studies supported the diagn of a defibrination syndrome. Antihae philic A factor proaccelerin and p thrombin values were significantly low ed while the antihaemophilic B and factors showed normal values. A decre of proactivator of the fibrinolytic syst was also found and should probably included in the defibrination syndro (4) Anticoagulant treatment was fo lowed by a considerable rise in t platelet count, and the fibrinogen lev also remained higher after transfusion fibrinogen than previously Antihaem philic A factor increased from less tha 60 per cent before treatment to abov 100 per cent (fig 2) At the same time the external bleeding from the surgica wound stopped

Theoretically an in vivo coagulation may take place in two ways 1 As a general process within the circulating

blood 2. As a local defibrination like that occurring in the retroplacental space after premature separation (14) We have no data which favours one of these possibilities more than the other

During anticoagulant therapy the TT value fell to less than 5 per cent, and this probably provoked the haemothorax. The reduction of phenylindanedione with subsequent increase in TT value, may explain the recurrence of the defibrination syndrome with fall in platelet count and fibrinogen which occurred during the last days before death.

One will always feel reluctant to give anticoagulant treatment to a patient with a severe bleeding tendency but from previous experience in one patient (5) who also gave satisfactory response on such therapy we believe that it might be the most adequate treatment in patients suffering from the defibrination syndrome. The main problem is to maintain a TT level which blocks the pathological coagulation process without impairing haemostasis. The effective therapeutic level in this situation is presumably somewhat lower than for anticoagulant therapy in other conditions. For this reason we tried t maintain a TT level of 10 per cent instead of 15 per cent.

Summary

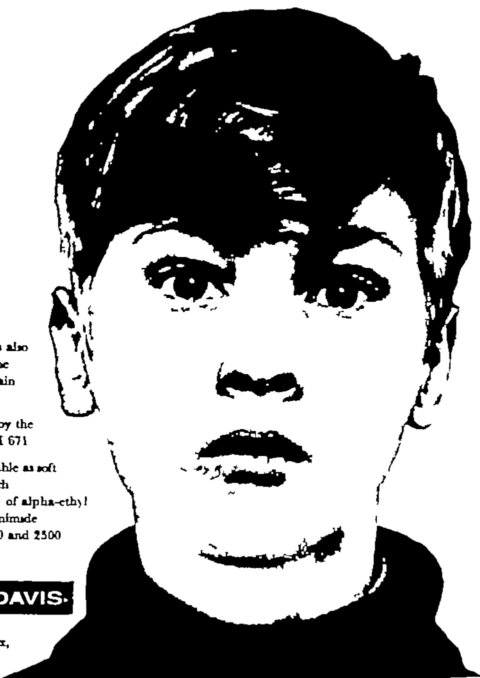
A patient with malignant intramuscular haemangioma n the left hip region is reported. Severe local bleeding in the place of the haemangioma occurred Detailed clotting studies gave evidence for the presence of a continuous coagulation process in vivo resulting in fibrinogenopenia and thrombocytopenia (the defibrination syndrome) This view was supported by the effect of anticoagulant treatment.

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Acta Medica Scandinavica. Vol. 173, fasc. 4, 1963

From the Departments of Medicine II and III, University of Helsinki, and from the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland

Benziodarone (Amplivix®) and Anticoagulant Therapy

By

Kalevi Pyörälä, Eero Ikkala and Pentti Siltanen

Benziodarone (Amplivix®) is a coronary dilator drug introduced by Charlier in 1959 (2). It is chemically 2-ethyl-3-(3',5' diiodo-4 hydroxybenzoyl)-benzofurane and remotely related to coumarin anticoagulants. According to Gillot (3) benziodarone in therapeutic dosage has no effect on blood coagulation and does not alter the prothrombin level of patients receiving bishydroxycoumarin. During clinical trials of benziodarone we were surprised to find that bleeding complications occurred, when benziodarone was given to patients receiving anticoagulant therapy with warfarin sodium. This finding prompted us to a closer study of the interference of benziodarone with anticoagulant therapy. The experiments reported in this paper were designed to study the effect of benziodarone on the response to various coumarin and indanedione anticoagulants.

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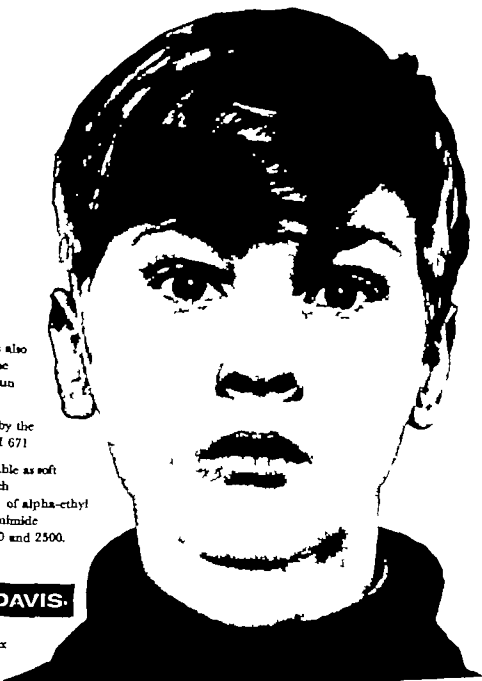
Material and methods

The effect of benziodarone on the response to various anticoagulants was studied in 90 patients in hospital with coronary heart disease who received anticoagulants for therapeutic purposes. The age of the patients ranged from 34 to 73 years. 78 of them were males and 12 were females. The patients were free from known liver, kidney or gastrointestinal disease and none of them had congestive heart failure. During the study they were on a regular hospital diet. Benziodarone was administered orally in 71 cases. In four cases it was given intravenously and in four cases rectally. The effect of benziodarone on the response to a single oral dose of warfarin sodium was studied in 12 healthy volunteers. All of them were males and their ages ranged from 23 to 35 years.

Owren's P & P method was used in the clinical control of anticoagulant therapy. In some experiments the coagulation mechanism was subjected to more thorough analysis including determination of the clotting activity by Quick's one-stage method, by Owren's P & P and thrombotest methods, and determination of prothrombin, factor VII factor IX,

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Table I The effect of benziodarone on the maintenance dosage of coumarin and indanedione anticoagulants

Anticoagulant	No. of patients	Anticoagulant dosage during control period (mg) Mean ± S. E.	Change of dosage during benziodarone (mg) Mean ± S. E.
Monocoumarins			
Warfarin sodium	15	7.4 ± 0.6	− 3.4 ± 0.1
Nicoumalone	7	4.4 ± 0.3	− 1.1 ± 0.3
Phenylpropylhydroxycoumarin	8	2.4 ± 0.4	− 0.1 ± 0.1
Dicoumarins			
Bishydroxycoumarin	9	50 ± 6	− 3 ±
Ethyl biscoumacetate	9	430 ± 47	−71 ± 23
Indanediones			
Phenylindanedione	10	103 ± 11	+ 2 ± 5
Chlorphenylindanedione	5	2.9 ± 0.4	+ 0.1 ± 0.1
Diphenylacetylindanedione	8	5.3 ± 0.8	− 2.2 ± 0.4

$0.02 > P > 0.01$ $P < 0.001$

and factor X. Factor X was determined by the method of Bachmann et al. (1) other methods used in this study have been described in an earlier paper (4)

Experiments

I The effect of benziodarone on the maintenance dosage of coumarin and indanedione anticoagulants

Anticoagulant drugs included in the study and the number of patients treated with each anticoagulant are shown in table I After stabilization of the anticoagulant dosage during 2—3 weeks benziodarone was given to the patients in the following dosage 200 mg three times a day for two days and thereafter 100 mg three times a day An attempt was made to maintain the P & P values on the same level as before the benziodarone treatment by adjustments of the anticoagulant dosage.

The effect of benziodarone on the maintenance dosage of the eight anticoag[illegible] studied is shown in table I

Benziodarone treatment had no definite effect on the dosage of phenylpropylhydroxycoumarin, bishydroxycoumarin, phenylindanedione, and chlorphenylindanedione. Anticoagulant effect of warfarin sodium, nicoumalone, ethyl biscoumacetate, and diphenylacetylindanedione was significantly increased. To maintain the same P & P level as before the benziodarone treatment, the maintenance dosage of warfarin sodium had to be reduced on an average by 46 % (fig 1) The average reduction of the dosage of nicoumalone was 25 % of the initial dosage. The corresponding figure for ethyl biscoumacetate was 17 % and for diphenylacetylindanedione 42 %.

Intravenous administration of benziodarone in a daily dosage of 300 mg to four patients receiving maintenance therapy with warfarin sodium was found to increase the effect of the anticoagulant. Similarly the effect of warfarin sodium was increased when benziodarone at the same dosage was given rectally to four patients.

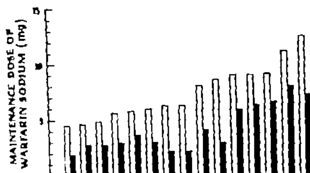


Fig. 1 The effect of benziodarone on the maintenance dosage of warfarin sodium in 15 cases. White columns present the mean daily dosage of warfarin sodium in each case during the control period and black columns show the mean daily dosage of warfarin sodium during benziodarone treatment.

II The effect of benziodarone on various coagulation factors in patients receiving anticoagulant therapy

Four patients, who were well stabilized on the maintenance therapy with warfarin sodium, were given 300 mg of benziodarone daily for 4 days. The dosage of warfarin sodium was left unaltered during the experiment. As shown in fig 2, the P & P values dropped below 10 % in all cases. Thrombotest method gave similar results. Determination of the four coagulation factors affected by anticoagulant therapy revealed a significant decrease of prothrombin, factor VII and factor X alterations of factor IX were not uniform.

In a similar experiment administration of benziodarone to three patients receiving bishydroxycoumarin and to four patients receiving phenylindanedione caused no corresponding changes in the various coagulation factors.

III The effect of benziodarone on the response to a single dose of warfarin sodium

The purpose of this experiment was to study the effect of benziodarone on the response of various coagulation factors to a single dose of warfarin sodium. Seven healthy subjects were given a test dose of 20 mg of warfarin sodium. Blood samples for coagulation studies were taken before the administration of the test dose and 24 and 48 hours after it. After 12 days new blood samples were taken and thereafter benziodarone treatment was started at a dosage of 600 mg a day Blood samples were taken 2 and 7 days after the beginning of the benziodarone treatment in order to reveal, if benziodarone given alone has any effect on the coagulation mechanism. Twenty mg of warfarin sodium was again given and blood samples were taken after 24 and 48 hours.

The results of the experiment are shown in fig 3 The response to the test dose of warfarin sodium was altered during the benziodarone treatment. The depression of prothrombin occurred earlier and was more pronounced The depression of factor VII was also increased, but the response of factor IX and factor X was unchanged. Benziodarone alone seemed to have no definite effect on the activity of various coagulation factors.

In a similar experiment five healthy subjects were given 300 mg of benziodarone daily This dosage of benziodarone also increased the response of prothrombin to the test dose of warfarin sodium, but did not modify the response of factor VII factor IX, and factor X.

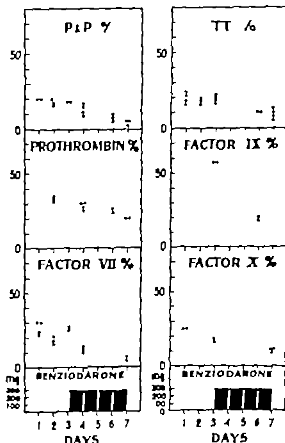


Fig 2. The effect of benziodarone on the P & P and thrombotest values and on the level of prothrombin, factor VII factor IX, and factor X in four patients receiving maintenance therapy with warfarin sodium.

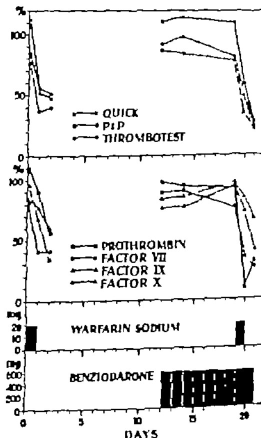


Fig 3 The effect of benziodarone treatment on the response to single dose of warfarin sodium.

Comments

Benziodarone given alone in therapeutic dosage seems to have no definite effect on blood coagulation This agrees with the finding that the response to phenylpropylhydroxycoumarin bishydroxycoumarin phenylindanedione, or chlorphenylindanedione is not altered during the benziodarone treatment. The increased response to warfarin sodium nicoumalone ethyl biscoumacetate, and diphenylacetylindanedione during the benziodarone treatment is an unexpected phenomenon, the mechanism of which remains unsolved so far Probably benziodarone, which is chemically related to anticoagulant drugs, interferes with the metabolism of the four anticoagulants mentioned and retards their detoxification or elimination from the body

Administration of benziodarone to patients receiving anticoagulant therapy with warfarin sodium, nicoumalone, ethyl biscoumacetate, or diphenylacetyldanedione results readily in bleeding complications, if the dosage of the anticoagulant drug is not properly reduced. Benziodarone can however well be given to patients receiving these anticoagulants, if the anticoagulant dosage is carefully stabilized during benziodarone treatment. Prolonged simultaneous administration of benziodarone and anti-

coagulants does not cause any unusual difficulties we have given benziodarone and warfarin sodium to three patients for more than one year without complications.

Summary

The effect of benziodarone (Amplivix®) on the response to various coumarin and indanedione anticoagulants was studied in patients on anticoagulant therapy. Administration of 300 mg of benziodarone daily did not alter the response to phenylpropylhydroxycoumarin, bishydroxycoumarin, phenylindanedione, or chlorphenylindanedione. The response to warfarin sodium, diphenylacetylindanedione, nicoumalone, and ethyl biscoumacetate was significantly increased during the benziodarone treatment. The dosage of warfarin sodium had to be reduced on an average by 46 per cent to maintain the clotting activity determined by the P & P method on the same level as before the benziodarone treatment. The corresponding figure for diphenylacetylindanedione was 42 per cent, for nicoumalone 25 per cent and for ethyl biscoumacetate 17 per cent.

Administration of benziodarone to patients receiving maintenance therapy with warfarin sodium was found to cause in a few days a fall of the P & P values below 10 per cent, if the anticoagulant dosage was left unchanged. Determination of various coagulation factors revealed a depression of prothrombin factor VII and factor X during the benziodarone treatment. The response to a single dose of warfarin sodium was also found to be increased during the benziodarone treatment.

Acknowledgements

Benziodarone (Amplivix®) used in this study was supplied through the courtesy of the Société des Laboratoires Labaz, Brussels, and Orion Oy Pharmaceutical Manufacturers, Helsinki. Nicoumalone (Sintrom®) and chlorphenylindanedione (Indaliton®) were kindly supplied by J. R. Geigy A. G., Basel.

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Distribution and Metabolism of Salicyl-azo-sulfapyridine

II. A Study with S^{35}-Salicyl-azo-sulfapyridine and S^{35}-Sulfapyridine

By

Å. Hanngren, E. Hansson, N. Svartz and S. Ullberg

The present investigation is the later part of a study concerning the distribution and metabolism of salicyl azo-sulfapyridine (salazopyrin® or azulfidine®) (SAP). In a previous work (4) we studied the distribution and metabolism of SAP and one of its metabolites 5-amino-salicylic acid (5-ASA) both labelled with C^{14} in the carboxyl group of 5-ASA.

We now present the results from a similar distribution and metabolism study with SAP and the other of its main metabolites, sulfapyridine, both labelled with S^{35} in the sulfapyridine.

The distribution was studied by whole-body autoradiography with sections of mice sacrificed at various intervals after administration. Radioactive products in the urine, liver and intestines were analyzed using paper chromatography.

Methods

Preparation of S^{35}-sulfapyridine (6)

S^{35}-sulphanilic acid supplied by the Radiochemical Centre, Amersham, was used. This was titrated to neutrality with aqueous sodium hydroxide solution and the freeze-dried sodium salt was acetylated with acetic anhydride. The sodium S^{35}-N-acetylsulphanilate was then ground with phosphorus pentachloride to give S^{35}-N-acetylsulphanilyl chloride which was reacted with 2 aminopyridine to give S^{35}-N-acetylsulphapyridine. This product was hydrolyzed with alkali to give S^{35}-sulphapyridine which was recrystallized from acetone. The specific activity calculated from the synthetical yields, was 54 μC/mg.

Preparation of S^{35}-salicyl-azo-sulphapyridine (6)

S^{35}-sulphapyridine was diazotized and coupled with salicylic acid in alkaline solution. The reaction product was precipitated with hydrochloric acid and washed several times with warm distilled water. The specific activity calculated from the synthetical yields, was 33 μC/mg.

Autoradiographic studies

The animal material consisted of 20 adult white mice, ten for each preparation studied (five male and five pregnant female in a late stage of gestation).

The compounds were injected intravenously and the animals in each series were sacrificed at 5 and 20 minutes, 1, 4 and 24 hours after injection. One series was given S^{35}-sulfapyridine in a dose of 0.01 mg (0.54 μC) per

Submitted for publication August 31, 1962.

Table I. A summary of the main autoradiographic findings for salazopyrin (SAP) labelled with C^{14} and S^{35} and its two main moieties C^{14} 5-amino-salicylic acid (C^{14} 5-ASA) and S^{35} sulfapyridine. The quantitative comparison is only relative for each tissue. The tissues are not compared to one another.

Tissue or organs considered	Comparison between the relative concentrations of radioactivity for			
	C^{14} 5-ASA	C^{14}-SAP	S^{35}-SAP	S^{35}-sulfapyridine
Activity in				
Blood 1 h	++	+++	+++	[1]+++
Blood 4 h	(+)	+	++	++
Blood 24 h	–	–	–	(+)
Connective tissue	+++	+++	++	–
Cartilage	++	–	–	–
Peritoneal, pleural and synovial fluids	+++ (higher than blood)	+++ (higher than blood)	++	(+) (lower than blood)
Vaginal secretion	–	+++	+++	+
Mammary glands	++	–	–	–
Gastric lumen	–	–	–	++
Intestinal lumen	++	+++	+++	(+)
Passage to				
Fetus	+++ (1 g time)	–	+	+++ (immediate penetrance)
CNS	–	–	+	+++ (immediate penetrance)
Disappearance from				
Tissues	++++ rapid	+++	++	+ slow

[1] Radioactivity of the blood was always higher than that of the tissues except excretory organs.
[2] Free sulfapyridine or its metabolites?

gram body weight and one series S^{35}-SAP in a dose of 0.01 mg (0.33 μC) per gram body weight.

The autoradiographic technique was the same as previously described (4) and the exposure time was about 3 weeks for both substances.

Chromatographic studies

To study the metabolism of S^{35}-sulfapyridine and S^{35}-SAP the same methods were used as previously for C^{14} 5-ASA and C^{14} SAP (4). Thus the urine of mice was investigated during a 24-hour period after intravenous and oral administration of the compounds. Further the metabolites in the liver and the intestines were studied 1 and 4 hours after intravenous and oral administration of S^{35}-SAP. The doses given were the same as for the autoradiographic studies.

The urinary excretion of radioactivity was investigated in 7 mice injected intravenously and 4 mice given orally S^{35}-SAP and in four mice injected intravenously and two mice given orally S^{35}-sulfapyridine. The mice were kept in metabolism cages and the urine samples were taken 1, 4 and 24 hours after administration of the compounds.

The urine was chromatographed directly on paper while the liver and intestines after homogenization were first extracted with water at pH 8 and then with ethanol before the pooled samples were put on chromatographic paper. Whatman no. 1 paper was used and the chromatograms developed by descending chromatography. The developing system was pyridine-isoamyl alcohol and water (35 : 35 : 30 v/v) and the spots were detected by ultraviolet light. Autoradiograms were made with X-ray film (Industrex, Kodak).

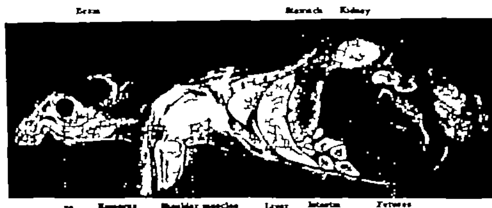


Fig. 1 Autoradiogram showing distribution of S^{35}-sulfapyridine in pregnant mouse 5 min. after intravenous injection. The radioactivity is rather evenly distributed and has already entered into the fetuses and brain. Signs of commencement of excretion through the gastric mucosa. No signs of connective tissue affinity

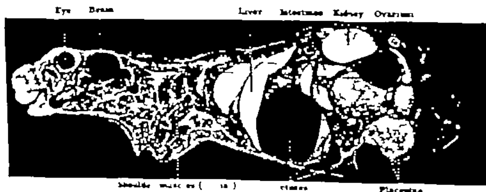


Fig. 2 Autoradiogram showing distribution of S^{35}-salicyl-azo-sulfapyridine (SASP) in pregnant mouse 5 min. after intravenous injection. No penetration to fetuses and brain. High concentration of radioactivity in the intestinal lumen. Note the affinity to connective tissue such as muscle fasciae.

Semiquantitative analyses was made by cutting out areas of the chromatograms and counting in liquid scintillation spectrometer (Tri-carb, Packard).

Results

A. Tissue distribution

In table I some autoradiographic findings are listed both for the two compounds dealt with specifically in this paper and for the two which were investigated previously — C^{14}-5-ASA and C^{14}-SAP (4).

Overall distribution data are thus summarized for salicyl-azo-sulfapyridine labelled in the sulfapyridine part, and in the 5-amino-salicylic acid part, and these are compared with similar data for the simple moieties.

A more detailed report concerning S^{35}-sulfapyridine and S^{35}-salicyl-azo-sul-

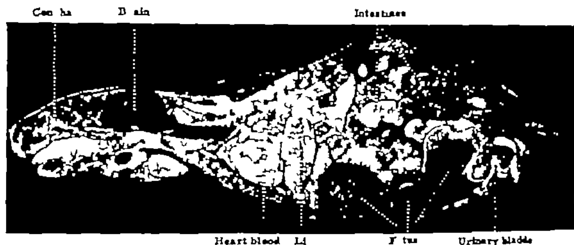


Fig 3 Autoradiogram of S^{35}-sulfapyridine 20 min. after injection into pregnant mouse. Very even distribution in the whole animal including the fetuses. No radioactivity in the intestinal lumen with exception of a small intestinal loop near the orifice of the common bile duct.

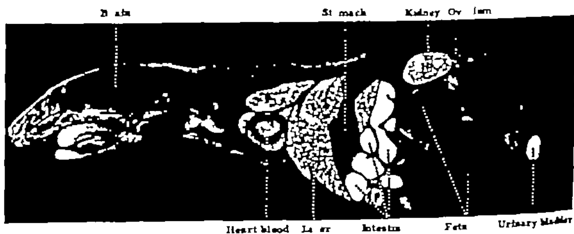


Fig 4 Autoradiogram of S^{35}-SAP 20 min after intravenous administration to pregnant mouse. Penetration to fetuses and brain hindered. Very high concentration in the intestines. No signs of excretion through the gastric mucosa.

fapyridine is presented below. Some autoradiographic findings with these substances are presented in fig 1—6.

1 S^{35} sulfapyridine

The distribution pattern of sulfapyridine was found to be very unlike that of the other substances studied in this series (5-ASA and SAP labelled with C^{14} or S^{35}), being much more even and showing longer maintenance of the blood and tissue concentrations. The blood concentration was always higher than the tissue concentrations, accumulation in tissues compared with the blood being found only in excretory organs, kidney and liver. Thus no specific binding of sulfapyridine to connective tissue or cartilage was observed.

Sulfapyridine apparently entered into body fluids such as peritoneal, pleural and synovial fluids. The concentration in

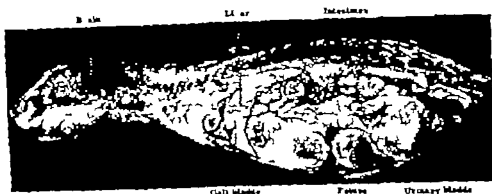


Fig. 5. Autoradiogram of S^{35}-sulfapyridine 1 hour after intravenous administration to pregnant mouse. Note the very even distribution in all tissues including fetuses and CNS. Accumulation in gall bladder.



Fig. 6. Autoradiogram of S^{35}-SAP 1 hour after intravenous administration to pregnant mouse. Accumulation in aortic wall, liver, gall bladder and intestines. Slight penetration to fetuses. Low activity in brain.

these fluids was, however, always found to be lower than in the blood, in contrast to what was found for C^{14}-5-ASA and S^{35}- and C^{14}-SAP. The passage to the brain and the fetuses also seemed to be free. Already five minutes after injection the brain and the fetal tissues showed significant S^{35}-concentrations and after 20 minutes they had reached approximately the same levels as most other tissues. Signs of excretion via the gastric but not the intestinal mucosa were observed 5 minutes after injection. The accumulation of radioactivity in the intestinal lumen was very low compared with the other substances studied.

2. S^{35}-salicyl-azo-sulfapyridine

The autoradiographic pattern of S^{35}-SAP is, especially in animals killed up to one hour after injection, very similar to that obtained for C^{14}-SAP.

Table II Semiquantitative values for different radioactive products found on paper chromatograms of urine after intravenous and oral administration of S^{35} sulfapyridine and S^{35}-SAP

Compound	R_f value	S^{35}-sulfapyridine						S^{35}-SAP					
		Intravenous (%)			Oral (%)			Intravenous (%)			Oral ( )		
		1 h	4 h	24 h	1 h	4 h	24 h	1 h	4 h	24 h	1 h	4 h	24 h
X_3	0.10—0.15	3	3	0	7	7	6	4	10	9	6	10	9
Acetylsulfapyridine	0.33—0.37	62	70	76	63	62	66	9	33	48	21	29	49
X_2	0.53—0.55	1	1	2	4	3	1	0	0	5	0	0	4
X	0 63—0.66	7	3	2	6	4	3	0	0	12	0	0	13
SAP	0.64—0.66	—	—	—	—	—	—	80	43	0	52	26	0
Sulfapyridine	0.82—0.86	27	23	20	20	24	24	7	14	26	21	25	25
	Total	100	100	100	100	100	100	100	100	100	100	100	100

A minor proportion possibly the metabolite X_1

Thus an accumulation in connective tissue was obtained which was marked 5 minutes and 20 minutes after injection, and could be observed also after 1 hour but had disappeared after 4 hours

As for C^{14}-SAP an accumulation was also noticed in the liver in the peritoneal, pleural and synovial fluids, in the endometrium and vaginal secretion. The accumulation in the lungs which was found for C^{14}-SAP was however less pronounced for S^{35}-SAP

As for C^{14} SAP a rapid and intense accumulation of radioactivity in the intestinal lumen was obtained

The passage to the CNS and through the placenta was hindered up to one hour after injection After four hours signs of passage to the brain and fetus were obtained and at this time the distribution picture showed a rather strong resemblance to that of S^{35}-sulfapyridine.

B. METABOLISM

Chromatograms of urine from mice given S^{35}-sulfapyridine intravenously and orally revealed five different radioactive products two of which could be identified as free sulfapyridine and acetylated sulfapyridine. The three other products, here called X_{1-3}, appeared in negligible quantities and were not analyzed The R_f-values of the constituents were sulfapyridine = 0 82—0 86 X_1 = 0 63—0.66, X_2 = 0.53—0.55 acetylsulfapyridine = = 0 33—0.37 and X_3 = 0 10—0 15 According to Williams (10) two of these metabolites, probably X_1 and X_3, may be hydroxylation products of sulfapyridine coupled to glucuronic acid.

All five spots were invariably found in urine collected at 1 and 4 hours, but X was sometimes absent in urine collected at 24 hours. In table II it can be seen that at 1 hour free sulfapyridine does not constitute more than about 30 per cent of the excreted radioactivity and that it thereafter shows a slight further decrease. The dominant metabolite is acetylated sulfapyridine the proportion of which progressively increases from about 60 to 75 per cent during the 24 hours of observation.

Urine from mice given S^{35}-sulfapyridine orally contains the same metabolites

in about the same proportions at the different times observed. The percentage of the product X_4 is however greater but it can still be considered a minor metabolite.

Chromatograms of urine from mice given S^{35}-SAP show apart from the parent substance sulfapyridine metabolites (table II). The main part of the radioactivity in 1-hour urine is SAP (80 %). Sulfapyridine and its acetyl conjugate appear in about equal proportions and constitute about 16 per cent of the total radioactivity. The minor metabolite X_4 has the same R_f-value as has SAP thus making it impossible to distinguish these substances as long as SAP is present. Comparing the 1-hour urine from mice given C^{14}-SAP where SAP also constitutes almost 80 per cent of the radioactivity the metabolite X_4 in the S^{35}-SAP experiments must be negligible at that time.

While SAP progressively decreases and cannot be found in the 24-hour urine, acetylsulfapyridine increases and becomes the main constituent. Free sulfapyridine slowly increases. The minor sulfapyridine metabolites (X_{1-4}) form a greater amount of relative radioactivity after SAP than after sulfapyridine administration. With oral administration the same metabolic course can be seen, though more rapid. Semiquantitative values for the different compounds are given in table II.

The intestinal radioactivity is exclusively unchanged SAP at one hour after administration of S^{35}-SAP while the liver at that time shows small amounts of free sulfapyridine and acetylated sulfapyridine. Four hours after S^{35}-SAP administration SAP is still the main radioactive compound in the intestines though very small amounts of sulfapyridine and acetylsulfapyridine are present.

Discussion

The results of the whole investigation concerning the two SAP-compounds and their moieties will be considered.

It is clear from the metabolic investigation that the salicyl-azo-sulfapyridine molecule gradually is split in the N = N linkage but that the radioactivity up to one hour after injection is present mainly as the unsplit SAP molecule. Thereafter up to ten different radioactive compounds can be seen on chromatograms from urine.

The sulfapyridine is excreted partly as sulfapyridine and partly as four probably conjugated products, the greatest part being acetylated sulfapyridine. 5-amino-salicylic acid is excreted as such only to a small extent but mainly as the acetylated form and to a smaller extent as two unidentified metabolites.

If the autoradiographic distribution pictures obtained after injection of the C^{14}-SAP and the S^{35}-SAP are compared with each other a close coincidence is observed up to one hour after injection. Later on, differences are apparent. The S^{35}-SAP picture now resembles the S^{35} sulfapyridine picture. The C^{14}-SAP picture however does not so closely resemble the C^{14} 5-ASA autoradiograms, probably because of the rapid excretion of 5-ASA and its metabolites.

If the autoradiographic pattern of the complex SAP molecule is compared with the pattern of its two moieties, the question arises as to what extent the distribution of SAP is influenced by its components.

As sulfapyridine does not show any specific tissue affinities and as its distribution does not seem to be blocked by barriers, it lacks characteristic distribution features. The affinity to connective tissue,

the high concentration found in peritoneal, pleural and synovial fluids, and the rapid and pronounced accumulation in the intestine are common to 5-ASA and SAP.

Concerning the connective tissue affinity of 5-amino-salicylic acid may be mentioned that 4-amino-salicylic acid (PAS) also shows a similar tendency (2) while this is not the case for salicylic acid (3). It thus looks as if the amino group can be held responsible for the connective tissue affinity of the amino-salicylic acids.

Helander (5) has in fluorescence microscopic investigations found connective tissue affinity for prontosil but not for sulfanilamide and for SAP but not for sulfapyridine. The N = N linkage which is common to both prontosil and SAP may therefore be considered as the possible carrier of the capacity of connective tissue affinity of the SAP molecule (7, 8).

A specific accumulation in endometrium and vaginal secretion was found for SAP but not for its simple components. It should however be pointed out that there are regions to which both the components of SAP have access but to which the penetration of SAP is hindered. Both S^{35}-sulfapyridine and C^{14} 5-ASA thus readily passed the placental barrier which was not the case for SAP.

The specific accumulation of C^{14}-5-ASA in the mammary gland and in cartilage was not noticed for either of the labelled SAP preparations.

A definite interpretation of the therapeutic significance of the present study must be postponed until the mechanism of action of the compound has been clarified in detail. One possibility is that the sulfapyridine moiety is the only active part. The combination of sulfapyridine with 5-amino-salicylic acid may then have the effect that the combined molecule is specifically accumulated in certain sites and that the sulfapyridine is continuously released from the sites of accumulation.

But an effect of 5-amino-salicylic acid similar to that reported for other salicylates on the metabolism of mucopolysaccharide compounds in connective tissue should also be considered (1, 9).

The therapeutically beneficial effect from the combination of the sulfapyridine and 5-amino-salicylic acid may be due to a retarded inactivation of the components through metabolic changes — mainly acetylation.

Summary

The second part of an investigation of the distribution and metabolism of the complex compound salicyl-azo-sulfapyridine (SAP) is reported.

Altogether four labelled compounds have been studied. The different compounds are SAP labelled with C^{14} in the COOH group of one of its moieties 5-amino-salicylic acid (5-ASA), SAP labelled with S^{35} in the other of its main moieties sulfapyridine, and the two simple labelled components.

The investigation has for each labelled compound comprised an autoradiographic distribution study of sections through whole mice, killed various times after the application of a single dose and a paper chromatographic analysis of the radioactive products in urine, liver and intestine.

Previously C^{14}-SAP and C^{14} 5-ASA have been studied. The present paper is specifically concerned with S^{35}-SAP and S^{35}-sulfapyridine.

Up to one hour after injection of C^{14}- or S^{35}-SAP the radioactivity mainly represents unsplit SAP but the SAP

molecule is gradually split in the azo linkage, and 5-amino-salicylic acid and sulfapyridine and metabolites — mainly conjugation products — are formed.

The S^{35}-sulfapyridine was distributed very evenly while the S^{35}-SAP (like C^{14}-SAP and C^{14}-5-ASA) showed affinity to connective tissue and was present in high concentration also in peritoneal, pleural and synovial fluids, in the liver and in the intestinal lumen.

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Protein-losing Enteropathy in Constrictive Pericarditis

By

V. Porsborg Petersen and J. Hastrup

The occurrence of hypoproteinaemia in cases of constrictive pericarditis has been attributed to such factors as malnutrition, repeated tappings of ascitic fluid and reduced liver function due to chronic venous congestion. Recently it has been reported that hypoalbuminaemia in constrictive pericarditis may be caused by an abnormal loss of serum albumin into the gastrointestinal tract (4, 5, 9). Davidson et al. (4) reported 3 cases in which hypoalbuminaemia and the abnormal intestinal loss of albumin disappeared after surgical treatment of the constriction had been performed and the venous pressure had returned to normal. The mechanism responsible for the increased intestinal loss of protein might consist in congestion of the intestinal wall due to elevated venous pressure and an excessive filtration of fluid from the capillaries into the lumen of the gut. An alternative explanation has considered the possibility of a disorder of the intestinal lymphatic apparatus, partly by analogy to the condition of "idiopathic hypoproteinaemia" with protein-losing gastroenteropathy, in which such disorder has been established (19).

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In this paper we report a case of constrictive pericarditis associated with gastrointestinal protein loss, in which a disorder of the intestinal lymphatic system was demonstrated by studies of thoracic duct lymph during life, and later by autopsy which showed anatomical lesions of the intestinal and mesenteric lymphatic vessels.

Case report

A 23-year-old man was admitted to this department in 1937 complaining of dyspnoea, ascites and oedema of one year's duration. Physical findings included enlargement of the liver, bilateral pleural exudates, ascites and oedema of the legs. The electrocardiogram showed low voltage and inversion of T-waves, and the venous pressure was 300 mm. Pericardiectomy was carried out in Sept. 1938. The pericardium was adherent and thickened to a 1—2 mm fibrous layer, of which pieces covering the anterior surface of the heart were removed. The clinical condition remained, however, unchanged after the operation, and during the following 17 years the patient needed laparocentesis every few weeks as well as weekly injections of a mercurial diuretic, whereby fluid retention could be partly controlled. In 1954 he was readmitted for in-

recurrence of the constriction syndrome. The fluid retention was unaffected by mercurial diuretics, while combined treatment with a spirolactone (Aldactone) and chlorothiazide produced sodium diuresis followed by a weight loss of 9 kg. After discharge in April 1961 the patient was able to resume his work as customs officer. In June 1961 he suffered mild cerebral concussion in traffic accident, otherwise his condition remained essentially unchanged until Nov. 1961. From then on he experienced several episodes of diarrhoea associated with increasing weakness and exhaustion. H. was admitted for the last time in Feb. 1962 in a very poor condition, after spell of diarrhoea. Persistent vomiting and shortness of breath were present, and the patient expired after four days' stay in hospital.

Serum proteins

The variations in total serum-protein and serum-albumin are recorded in fig. 1. Analyses during 1954—55 were made by Kjeldahl estimations and fractionation by ammonium sulphate precipitation. Later analyses consist of paper electrophoresis according to Laurell et al. (14) and total protein by the method of Weichselbaum (20). Although analyses were done less frequently at the time of the second pericardiectomy it appears that hypoalbuminaemia was present before the operation, and that serum-albumin increased after surgical relief of the constriction. The low serum-albumin cannot be due to dilution since serum-globulin remained unchanged and the haematocrit was normal. When the patient was readmitted in 1961 hypoalbuminaemia was again present. Serum-albumin usually ranged between 1.1 and 1.4 g/100 ml. The globulins were within normal limits, except γ-globulin, which was at or slightly above the upper normal limit.

Albumin metabolism

Abnormal gastrointestinal permeability was demonstrated by testing with ^{131}I-labelled polyvinylpyrrolidone as described by Gordon (7). Cumulative intestinal excretion of radioiodine in 5 days was 9.2 of the injected dose (normal below 1 %).

Albumin turnover was measured by ^{131}I labelled human serum-albumin. Thyroidal uptake was blocked by administration of stable iodine. The degradation of labelled albumin was followed by daily measurements of plasma activity and of the rate of urinary excretion of radioiodine. Faecal activity was determined in homogenized stools made up to known volume. Radioactivity was measured in a well-type scintillation counter connected with a Tracerlab Superscaler. From these analyses albumin turnover was calculated according to the method described by Pearson et al. (16). From table I it appears that total exchangeable albumin was much reduced due to an increased degradation as indicated by high fractional turnover rate, shortened half-life and a high faecal excretion of the tracer. The absolute rate of albumin turnover was in the lower normal range, this indicating a limited capacity for albumin synthesis. Cases of "idiopathic hypoproteinaemia" with gastrointestinal protein loss are generally associated with an increased synthesis. In this respect only is the pattern of deranged albumin metabolism in this patient at variance with this condition. The reason for this difference in albumin synthesis capacity may be found in reduced liver function due to cardiac cirrhosis.

Table I. Albumin metabolism

	Patient	Normal[1]
Body weight (kg)	71	—
Serum-albumin (g/100 ml)	1.5	4.0—4.9
Total albumin (g/kg)	1.03	3.8—5.0
Circulating albumin (g/kg)	0.48	1.78—2.56
Extravascular albumin pool (% of total)	54	48—69
Albumin degradation (% of circulating albumin/day)	29	6—14
Albumin half-life (days)	2.4	5—12
Albumin turnover (mg/kg/day)	139	130—280
Faecal output of ^{131}I in 5 days (% of dose)	0.92	< 0.40

[1] Jarnum and Schwartz (10).

Serum proteins in gastrointestinal secretions

Immunoelectrophoresis was carried out on samples of digestive fluids obtained by intubation of the stomach, duodenum and je-

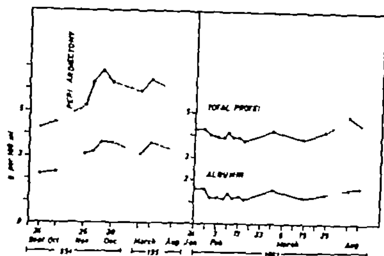


Fig 1 Variations in total serum-protein and serum-albumin.

vestigation with a view to a renewed attempt at surgical treatment.

At that time physical examination revealed moderate orthopnoea and considerable distension of the neck veins. Auscultation showed arrhythmia which was due to atrial fibrillation The liver was enlarged and bilateral hydrothorax, ascites and oedema to the groin were present. Right heart catheterization showed a right atrial pressure of 14/7 mm Hg and atrial and ventricular pressure curves with contours compatible with a diagnosis of constrictive pericarditis. At thoracotomy in Oct. 1954 extensive calcifications and thickened pericardium were excised the heart was thoroughly explored digitally and the entire pericardial lining except the diaphragmatic part was removed. The operation was followed by excellent clinical improvement. Fluid retention, hepatomegaly and venous distension disappeared and venous pressure was reduced to 120—130 mm. Postoperative heart catheterization showed, however only a moderate reduction in right-atrial pressure to 12/4 mm Hg which is well above normal

The patient remained in good health for the following 6 years. From June 1960 exertional dyspnoea and oedema of the legs reappeared, and at the same time he also began to have periodic diarrhoea. He was readmitted in Jan. 1961 His general condition was fairly good. There was no orthopnoea and venous distension was not present. The heart sounds were normal atrial fibrillation was present as previously There were light ascites and moderate oedema of the legs. Hydrothorax was present on the right side and at thoracocentesis 1 000 ml yellow clear serous fluid was removed. The venous pressure was 125 mm in the left external jugular vein. Arterial blood pressure was 120/70 mm Hg

Laboratory examinations showed a haemoglobin of 94 The white blood cell count was 6,700/μl, with a normal differential count. Serum electrolytes were normal, except serum-calcium, which was 8.0 mg/100 ml. Liver function tests were normal except for a positive thymol turbidity test. A pronounced hypoproteinaemia was found with a serum-albumin consistently below 2 g/100 ml. The urine did not contain protein.

Chest radiographs showed the heart to be of normal size and shape. Pericardial calcifications were seen at the diaphragmatic surface of the heart. X rays of the gastro-intestinal tract showed a normal stomach and large intestine. The proximal jejunal loops were slightly dilated and coarse mucosal folds suggested the presence of oedema.

Initially the patient had four to six bowel movements per day later only two. Stools were soft, occasionally semi-liquid Mild steatorrhoea was found the faecal fat content was 16 and 20 g per day on two occasions. The xylose-test was normal.

In view of the absence of venous distension and in the presence of a questionably elevated venous pressure which had remained unaltered since the pericardiectomy it was considered likely that fluid retention was mainly due to hypoalbuminaemia rather than to

recurrence of the constriction syndrome. The fluid retention was unaffected by mercurial diuretics, while combined treatment with a spirolactone (Aldactone) and chlorothiazide produced a sodium diuresis followed by weight loss of 9 kg. After discharge in April 1961 the patient was able to resume his work as customs officer. In June 1961 he suffered mild cerebral concussion in traffic accident, otherwise his condition remained essentially unchanged until Nov. 1961. From then on he experienced several episodes of diarrhoea associated with increasing weakness and exhaustion. He was admitted for the last time in Feb. 1962 in a very poor condition, after a spell of diarrhoea. Persistent vomiting and shortness of breath were present, and the patient expired after four days' stay in hospital.

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The variations in total serum-protein and serum-albumin are recorded in fig. 1. Analyses during 1954—55 were made by Kjeldahl estimations and fractionation by ammonium sulphate precipitation. Later analyses consist of paper electrophoresis according to Laurell et al. (14) and total protein by the method of Weichselbaum (20). Although analyses were done less frequently at the time of the second pericardiectomy, it appears that hypoalbuminaemia was present before the operation, and that serum-albumin increased after surgical relief of the constriction. The low serum-albumin cannot be due to dilution since serum-globulin remained unchanged and the haematocrit was normal. When the patient was readmitted in 1961 hypoalbuminaemia was again present. Serum-albumin usually ranged between 1.1 and 1.4 g/100 ml. The globulins were within normal limits, except γ-globulin, which was or slightly above the upper normal limit.

Albumin metabolism

Abnormal gastrointestinal permeability was demonstrated by testing with ^{131}I-labelled polyvinylpyrrolidone as described by Gordon (7). Cumulative intestinal excretion of radio-iodine in 5 days was 9.2 % of the injected dose (normal below 1 %).

Albumin turnover was measured by ^{131}I-labelled human serum-albumin. Thyroidal uptake was blocked by administration of stable

Table I. Albumin metabolism

	Patient	Normal[1]
Body weight (kg)	71	–
Serum-albumin (g/100 ml)	1.3	4.0—4.9
Total albumin (g/kg)	1.05	3.8—5.0
Circulating albumin (g/kg)	0.48	1.28—2.96
Extravascular albumin pool (% of total)	54	48—69
Albumin degradation (% of circulating albumin/day)	29	6—14
Albumin half-life (days)	2.4	5—12
Albumin turnover (mg/kg/day)	139	130—200
Faecal output of ^{131}I in 5 days (% of dose)	0.92	< 0.40

[1] Jarnum and Schwartz (10)

iodine. The degradation of labelled albumin was followed by daily measurements of plasma activity and of the rate of urinary excretion of radioiodine. Faecal activity was determined in homogenized stools made up to known volume. Radioactivity was measured in well-type scintillation counter connected with a Tracerlab Superscaler. From these analyses albumin turnover was calculated according to the method described by Pearson et al. (16). From table I it appears that total exchangeable albumin was much reduced due to an increased degradation as indicated by a high fractional turnover rate, shortened half-life and high faecal excretion of the tracer. The absolute rate of albumin turnover was in the lower normal range, this indicating limited capacity for albumin synthesis. Cases of "idiopathic hypoproteinaemia" with gastrointestinal protein loss are generally associated with an increased synthesis in this respect only is the pattern of deranged albumin metabolism in this patient at variance with this condition. The reason for this difference in albumin synthesis capacity may be found in reduced liver function due to cardiac cirrhosis.

Serum proteins in gastrointestinal secretions

Immunoelectrophoresis was carried out on samples of digestive fluids obtained by intubation of the stomach, duodenum and je-

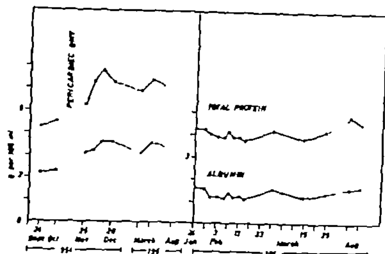


Fig 1 Variations in total serum-protein and serum-albumin.

vestigation with a view to a renewed attempt at surgical treatment.

At that time physical examination revealed moderate orthopnoea and considerable distention of the neck veins. Auscultation showed arrhythmia, which was due to atrial fibrillation. The liver was enlarged and bilateral hydrothorax, ascites and oedema to the groin were present. Right-heart catheterization showed a right atrial pressure of 14/7 mm Hg and atrial and ventricular pressure curves with contours compatible with a diagnosis of constrictive pericarditis. At thoracotomy in Oct. 1954 extensive calcifications and thickened pericardium were excised the heart was thoroughly explored digitally and the entire pericardial lining except the diaphragmatic part was removed. The operation was followed by excellent clinical improvement. Fluid retention, hepatomegaly and venous distension disappeared and venous pressure was reduced to 120—130 mm. Postoperative heart catheterization showed, however only a moderate reduction in right atrial pressure to 12/4 mm Hg which is well above normal

The patient remained in good health for the following 6 years. From June 1960 exertional dyspnoea and oedema of the legs reappeared, and at the same time he also began to have periodic diarrhoea. He was readmitted in Jan. 1961 His general condition was fairly good There was no orthopnoea and venous distension was not present. The heart sounds were normal atrial fibrillation was present as previously There were light ascites and moderate oedema of the legs. Hydrothorax was present on the right side and at thoracocentesis 1 000 ml yellow clear serous fluid was removed. The venous pressure was 125 mm in the left external jugular vein. Arterial blood pressure was 120/70 mm Hg

Laboratory examinations showed a haemoglobin of 94 The white blood cell count was 6 700/μl, with a normal differential count. Serum electrolytes were normal, except serum-calcium, which was 8.0 mg/100 ml. Liver function tests were normal except for a positive thymol turbidity test. A pronounced hypoproteinaemia was found with a serum-albumin consistently below 2 g/100 ml. The urine did not contain protein.

Chest radiographs showed the heart to be of normal size and shape. Pericardial calcifications were seen at the diaphragmatic surface of the heart X-rays of the gastrointestinal tract showed a normal stomach and large intestine. The proximal jejunal loops were slightly dilated and coarse mucosal folds suggested the presence of oedema.

Initially the patient had four to six bowel movements per day later only two. Stools were soft, occasionally semi-liquid. Mild steatorrhoea was found the faecal fat content was 16 and 20 g per day on two occasions. The xylose-test was normal.

In view of the absence of venous distension and in the presence of a questionably elevated venous pressure which had remained unaltered since the pericardiectomy it was considered likely that fluid retention was mainly due to hypoalbuminaemia rather than to

recurrence of the constriction syndrome. The fluid retention was unaffected by mercurial diuretics, while combined treatment with a spirolactone (Aldactone) and chlorothiazide produced sodium diuresis followed by a weight loss of 9 kg. After discharge in April 1961 the patient was able to resume his work as customs officer. In June 1961 he suffered a mild cerebral concussion in a traffic accident, otherwise his condition remained essentially unchanged until Nov. 1961. From then on he experienced several episodes of diarrhoea associated with increasing weakness and exhaustion. He was admitted for the last time in Feb. 1962 in very poor condition, after spell of diarrhoea. Persistent vomiting and shortness of breath were present, and the patient expired after four days' stay in hospital.

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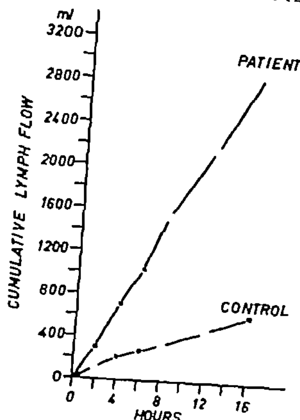


Fig 2 Thoracic duct lymph flow

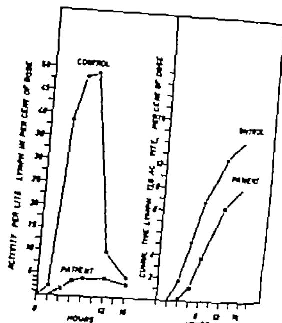


Fig 4 Activity per litr lymph (left) and cumulative transport of tracer after ingestion of ¹³¹I-oleic acid.

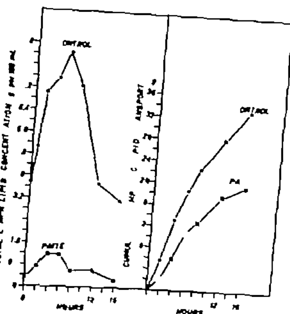


Fig 3. Concentrations of total lymph-lipid (left) and cumulative transport of lipid after ingestion of cream.

jejunum under X-ray control. Unconcentrated samples showed the presence of serum-albumin in fluid from each of these regions. After five-fold concentration by vacuum dialysis gastric juice was found to contain also α_2-haptoglobin, α_2-lipoprotein and traces of transferrin and γ-globulin. Duodenal fluid contained transferrin and jejunal fluid α_2-haptoglobin and traces of transferrin and γ-globulin.

Studies of thoracic duct lymph

Lymph was obtained after cannulation of the thoracic duct in the neck according to the technique described by Linder and Blomstrand (15) Under local anaesthesia an incision was made parallel to the clavicle and a plastic catheter inserted into the duct and held in place by a suture, without ligation of the duct. During the collection of lymph the patient was confined to bed. Food was withheld for the following 16 hours, while fluid was given ad lib 200 ml of cream with 18 % fat was administered with a tracer dose of 50 μC ^{131}I-labelled oleic acid (obtained from the Radiochemical Centre, Amersham) in gelatine capsules. Thyroidal uptake was blocked and radioactivity measured as outlined above. The flow-rate of duct lymph was

Table II Chemical composition of serum and of thoracic duct lymph in the fasting state and six hours after ingestion of cream

	Patient			Control subject		
	Serum fasting	Lymph		Serum fasting	Lymph	
		Fasting	6 hrs		Fasting	6 hrs
	mg%	mg%	mg%	mg%	mg%	mg%
Total lipid	723	353	1,175	772	5 710	8,465
Cholesterol	221	29	13	272	139	130
Phospholipid	187	57	122	260	336	580
Neutral fat	315	267	1,040	240	3,225	7 755
	g %	g %	g %	g %	g %	g %
Total protein	4.5	0.9	0.8	6.6	5.3	5.5
Albumin	1.6	0.2	0.2	4.4	3.8	4.0
α-globulin	0.9	0.2	0.2	0.8	0.6	0.4
β-globulin	0.7	0.2	0.2	0.8	0.4	0.4
γ-globulin	1.5	0.3	0.2	0.6	0.5	0.7
	%	%	%	%	%	%
α-lipoprotein	23	23	28	24	9	6
β-lipoprotein	77	77	74	76	91	94

recorded by continuous collection in 2 and 4-hour periods and additional samples were obtained at the end of each period. Chemical analyses for lipids, proteins and electrolytes were done by standard laboratory procedures. The results were compared with those obtained in control subject, 42-year-old female, in whom cannulation of the thoracic duct was performed following supraclavicular and mediastinal biopsy of lymph nodes. This patient had 2 × 4 cm large lesion in the left upper pulmonary lobe, without any other signs of disease. The lymph nodes removed were histologically normal, and the pulmonary lesion later proved to be benign.

The pressure in the thoracic duct in the horizontal position, as given by the height above the sternum of the lymph column in the catheter was 25 cm in the patient and 6 cm in the control subject.

Volumes of lymph during 16-hour experimental period following ingestion of the cream are recorded in fig 2. It appears that the lymph flow occurred at an almost constant rate in both subjects throughout the experimental period, but that the flow was almost 5 times as high in the patient as in the control subject. The average flow rate in the patient was 185 ml/hour against 40 ml/hour in the control. Normal flow-rates of thoracic duct lymph in humans are reported to be 0.4—1.6 ml/kg/hour (21). Related to body weight flow-rates were 3.1 ml and 0.6 ml in the patient and control respectively. Lymph from the control subject was thick and creamy while that from the patient was opalescent, watery and slightly haemorrhagic, with haematocrit of 2—4 %.

The concentrations and cumulative transport of lipid are recorded in fig 3. In both subjects lipid concentration rose after ingestion of cream, but large difference is apparent. In the control the lipid concentration reached a peak at 6 hours of over 8 g/100 ml, while in the patient it rose gradually to a level at about 1 g/100 ml at 4 and 6 hours. The absolute quantity of fat transported via the thoracic duct also showed a rather large difference in that 33 g were recovered after 16 hours in the control subject as com-



Fig 5 Swollen, oedematous intestine from patient (left) compared with normal intestine (right)



Fig 6. Lakes of lymph in jejunal submucosa. Villi swollen by dilated lymphatic vessels. Frozen-section. Sudan × 100

pared with 19 g in the patient. The fat content of the cream ingested was 36 g

The differences in lymphatic transport also appear from fig 4 which shows the time-course curves of activity per litre of lymph and the cumulative transport of activity as percentages of the dose administered. In addition to the large difference in concentration, which in the control rose to a peak of 50 %/l at 6 hours, there is also a difference in the shape of the curves. In the patient only traces of activity could be detected in the 2-hour sample, and lymph activity rose to a low plateau lasting from 6 to 16 hours after ingestion of labelled oleic acid. The difference in cumulative transport of the tracer is par



Fig. 7 Mesenteric lymphatic vessel filled up with lipophages. Lines indicate size. Sudan × 235

ticularly apparent during the first 6 hours, in which the control subject carried more than 5 times as much of the ingested activity as the patient.

The chemical composition regarding lipid and protein fractions in lymph and serum is shown in table II. In both cases lymph phospholipid concentration rose after the fat meal, while total cholesterol remained essentially unchanged. The protein content and particularly albumin concentrations were very low in the patient's lymph. Compared with serum, lymph-albumin was less than 15 %, while in the control lymph-albumin was close to 90 % of that in serum. The total amount of albumin returned by way of the thoracic duct within 16 hours was 4 1 g in the patient and 22.4 g in the control subject.

The patient died about 18 months after fluid retention had reappeared and about one year after completion of the studies reported above.

Autopsy

Extensive fibrous adhesions were present in the anterior mediastinum and in both pleural cavities. The left pleural cavity was obliterated, while the right contained 1 800 ml serous fluid The left internal jugular left subclavian and left innominate veins were somewhat narrowed by surrounding scar tissue, which extended upwards from a heavy oedematous areolar tissue in the upper mediastinum. The right innominate vein and the superior vena cava were of normal calibre, and the endothelial lining of all the large chest veins was normal.

The thoracic duct was isolated in its abdominal part and injected with India ink. No obstruction or kinking was observed. The duct took normal course through the diaphragm and chest and joined the venous system at the junction of the left internal jugular and subclavian veins. The terminal part was dilated, forming an ampullar structure, presumably resulting from the cannulation, although no traces of the ligature and no obstruction or narrowing of the opening into the venous angle could be discerned. There were no visibly dilated lymphatics in the mediastinal structures.

The heart was surrounded by white fibrous tissue, which contained calcifications in the diaphragmatic part. The myocardium was somewhat thin, otherwise normal. The endocardium and the heart valves were normal.

The peritoneal cavity contained 800 ml of chylous fluid. The entire peritoneal membrane was thickened, particularly at the lower diaphragmatic surface, which was covered by a 3—4 mm thick fibrous layer. The small intestine was heavy, oedematous, dilated and all intestinal loops were glued together by fibrous adhesions. The intestinal mucosa contained numerous dilated lymphatics in plexiform arrangement scattered along the entire length of the small intestine, but particularly prominent in the jejunum. The mesenterial lymph nodes were of normal size or slightly enlarged. The large intestine was normal. The liver was slightly shrunken and firm. The portal vein was of normal calibre and without obstructions. The pancreas, kidneys and adrenals were normal. The spleen was enlarged and surrounded by thick fibrous capsule. The brain was very oedematous with conus formation at the cerebellum.

Histology

The valves of Kerkring and the villi were enlarged and swollen due to expansion of the lymphatic vessels, which contained foamy lipophages. In paraffin-embedded sections these cells had light cytoplasm with many vacuoles and several nuclei. Frozen-section preparations showed large amounts of sudanophilic material in the cytoplasm, but no acid-fast, PAS-positive or fluorescent substances. In some areas dilated lymphatics extended down into the lamina propria and submucosa as a plexiform, angiomatous arrangement filled with lipophages. The lymphangiectasia was predominantly present in the jejunum, less so in the ileum, and absent in the stomach and the large intestine.

The dilated lymphatics could be followed into the mesentery and retroperitoneal tissue. The vessel walls were only slightly thickened and the elastica interna appeared normal. The mesenteric and retroperitoneal lymph nodes were surrounded by a thickened, fibrotic capsule, the trabeculae were enlarged and the medulla diffusely fibrotic. The sinuses were dilated and contained reticulum cells and lipophages. The thoracic duct was histologically normal.

The external muscularis of the intestinal wall contained a yellowish-brown pigment, which histochemically reacted as a lipofuscin. Examination of unstained sections in ultraviolet light showed fluorescence with a yellow light. Pigment granules were found in all sections of the small intestine, but mostly in the upper part, and they were absent in the colon. Small deposits were also found in striated muscle and in the myocardium. The pigment is in every respect similar to that found in cases of idiopathic hypoproteinaemia with protein-losing gastroenteropathy (11). Microscopy of the liver showed a mild portal cirrhosis.

Discussion

The mechanism underlying abnormal intestinal protein loss in cases not associated with gross anatomical lesions, such as gastric carcinoma, regional enteritis and ulcerative colitis has remained unknown until recently. Several lines of evidence have indicated, however, that abnormalities of the intestinal lymphatic apparatus might be involved. Laparotomy in cases of "idiopathic hypoproteinaemia" due to gastrointestinal protein loss has shown thickened and oedematous intestinal loops with dilated lymphatics and in some cases chylous effusions in the peritoneal cavity (8, 11).

The anatomical lesions affecting the internal lymphatic pathways in the

present patient with constrictive pericarditis are similar to those described recently by Waldmann et al. (19) in cases of idiopathic hypoproteinaemia and protein losing gastroenteropathy. Intestinal specimens revealed the presence of dilated lymphatic vessels in the mucosa and submucosa, sometimes forming plexiform enlargements causing distortions of the villi and containing foamy lipophages. Specimens obtained at autopsy or laparotomy showed a thickened and fragmented elastica interna and hypertrophy of media of the mesenteric lymphatics. These authors suggested the term "intestinal lymphangiectasia" for the disorder in which there is gastrointestinal protein loss and dilated lymphatic channels in the intestinal wall and mesentery of the small bowel. The intestinal loss of protein might be due to rupture of dilated lymphatics or to increased permeability and high intraluminal pressure with consequent escape of lymph from the mucosal vessels into the lumen of the gut and also from the serosal vessels into the peritoneal cavity. As in many cases of idiopathic hypoproteinaemia this patient also had mild steatorrhoea, which could be due to impaired fat absorption secondary to delayed intestinal lymphatic transport. An alternative explanation is afforded by the hypothesis just mentioned which would imply that the increased faecal fat is of endogenous origin i e the presence of a protein-and fat losing enteropathy with an identical mechanism for the loss of protein and of fat.

The studies of thoracic duct lymph now reported show that the intestinal lymphangiectasia was associated with a derangement of intestinal lymphatic function. Lymph from the gastrointestinal tract and from the liver normally comprises about 90 per cent of thoracic duct lymph, and the production of large volumes of lymph under a high pressure indicates a marked increase in the formation of lymph in tissues drained by the thoracic duct. In his classic study on the formation of lymph Starling (18) demonstrated that obstruction of the inferior vena cava above the diaphragm caused a sharp increase of flow in the thoracic duct of lymph originating in the liver and with a high content of solids. Obstruction of the portal vein also increased flow in the thoracic duct but the lymph now produced was of a different quality being more watery. Starling also noted that the rise of pressure in the portal vein and the intestinal capillaries caused haemorrhage per diapedesim and the presence of red cells in the lymph. The high protein content in liver lymph has later been amply confirmed (21). Cain et al. (3) studied the composition of liver lymph and thoracic duct lymph obtained separately in the dog and found that the protein content of liver lymph was about five-sixths that of plasma, while duct lymph contained approximately one half that of plasma. Protein content in thoracic duct lymph in humans with leukaemia was 70 to 90 per cent of that in plasma (1) and in the present control subject 80 per cent of that in plasma. The very low protein content in thoracic duct lymph from the patient suggests that the increased formation of lymph originated outside the liver and presumably in the small bowel.

An increased flow of thoracic duct lymph in constrictive pericarditis might result from a high pressure in the inferior vena cava and/or in the portal vein. The concept that constrictive pericarditis causes a derangement in the production and flow of thoracic duct lymph is supported by studies of experimental pericarditis in dogs. Blalock and Burwell (2)

produced constrictive pericarditis by introducing aleuronat into the pericardial cavity Three weeks later the thoracic duct was found dilated by lymph under a high pressure and on opening the duct copious amounts of lymph came out. Similar findings were reported by Földi et al. (6) who induced asbestos-pericarditis with hydrothorax and ascites in dogs and noted a four-fold increase in thoracic duct lymph-flow In four patients with constrictive pericarditis studied by us high flow rates were found in all cases 2 of these patients had normal serum-albumin and 2 were border line cases of gastrointestinal protein loss (17)

The causative factors responsible for intestinal protein loss in constrictive pericarditis might then include cardiac inflow-stasis with increased pressure in the inferior vena cava and the portal vein, increased intestinal capillary pressure causing increased production of lymph with secondary formation of dilated lymphatics, and loss of protein and fat by rupture or by escape of lymph by transudation under high pressure. It is conceivable that this sequence is potentially reversible by relief of the pericardial constriction which would explain the disappearance of i testinal protein loss after pericardiectomy

Other conditions in which a high venous pressure prevails over a prolonged time and which therefore might evoke a similar chain of events include cases of right-sided heart failure. Hypoalbuminaemia and intestinal protein was found n a case of interatrial septal defect with high venous pressure by Davidson et al. (4) and n a case of pulmonary stenosis by Jeejeebhoy (13) Portal hypertension might be expected to cause intestinal protein loss, but in the few cases of chronic liver disease examined so far this has not been demonstrated (12) One reason for this may be that a time interval must elapse before the intestinal lymphatics yield to elevated venous and capillary pressure.

In the case reported here a high venous pressure was present for 17 years before effective surgical therapy was performed. In retrospect t appears likely that temporary intestinal protein loss occurred previous to the second pericardiectomy as suggested by a low serum-albumin, which became normal after relief of the construction The later development of permanent hypoalbuminaemia and intestinal loss may be accounted for by the fact that although clinical improvement ensued and the patient remained free of oedema and ascites for six years, a slight cardiac inflow-stasis probably persisted during this period. Only a moderate reduction in right-atrial pressure, which remained above normal, was obtained, and venous pressure after the operation remained at the upper normal limit or slightly increased, at 120—130 mm. The combined effect of intestinal protein loss and impaired hepatic synthesis of albumin resulted in a very low serum-albumin, which per se will contribute to a large flow of thoracic duct lymph (6, 18) This means that a vicious circle may be established in which hypoalbuminaemia perpetuates high flow-rates of thoracic duct lymph and a high pressure system in the intestinal lymphatic apparatus leading to irreversible anatomical lesions as found in the present case.

Summary

A case of constrictive pericarditis associated with protein-losing enteropathy is described. Permanent hypoalbuminaemia developed several years after pericar

diectomy which had relieved clinical symptoms, but probably still left a slight cardiac inflow-stasis.

Increased gastrointestinal permeability was demonstrated by means of ^{131}I polyvinylpyrrolidone, and studies with ^{131}I albumin showed a reduced pool of exchangeable albumin and a high fractional turnover of serum-albumin.

Investigations of thoracic duct lymph showed a greatly increased production of lymph with a low protein content probably originating in the small intestine. The transport of fat after ingestion of cream was reduced and measurements of lymph radioactivity after oral ingestion of oleic acid labelled with ^{131}I indicated a delayed transport of the tracer from the intestinal lumen to the thoracic duct.

Autopsy findings revealed thickening and oedema of the small intestine and the presence of intestinal lymphangiectasies which are assumed to be secondary to a high pressure in the intestinal lymphatic pathways caused by increased production of lymph.

In cases of constrictive pericarditis this could be elicited by an increased pressure in the inferior vena cava and/or the portal vein, and perpetuated by a low serum albumin.

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We wish to thank Dr Robert S. Gordon, the National Heart Institute, Bethesda, Maryland, U S. A., and Dr Michael Schwartz, Amtssygehuset, Glostrup, Denmark, who supplied us with ^{131}I PVP and ^{131}I albumin, respectively The isotope measurements were carried out in the Radiophysics Department of the Radium Centre for Jutland, Aarhus. We are grateful to Dr C. B. Madsen for permission to use the facilities of the laboratory and to Dr Poul Ottosen of the Department of Thoracic Surgery Aarhus Kommunehospital, who performed the thoracic duct cannulations.

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The Triiodothyronine Suppression Test

By

H. P. Østergaard Kristensen, M. Dyrbye and L. Korsgaard Christensen

In most cases of thyrotoxicosis the diagnosis can be based on the clinical appearance combined with determination of the basal metabolic rate.

However the clinical features are often uncharacteristic and the BMR only slightly elevated or possibly normal. In such cases, determination of the protein-bound iodine in the serum (PBI ^{127}) and determination of the radioactive iodine uptake in the thyroid gland as well as of protein-bound I^{131} in the serum (PBI^{131}) following administration of I^{131} are useful (5). Moreover by supplementing these tests with determination of the erythrocyte uptake of triiodothyronine or determination of diffusible, free thyroxine, great diagnostic accuracy may be attained.

Nevertheless, there will be a few complicated cases in which a combined evaluation of the results of all these tests is not conclusive. This applies, for example, to cases of previous thyrotoxicosis treated with subtotal thyroidectomy or I^{131}. In these cases the I^{131} uptake as well as the PBI^{131} may be elevated. Moreover the clinical assessment is often difficult, especially if exophthalmos persists, as this *per se* lends the patients a thyrotoxic appearance. As a rule, these complicated cases represent very early thyrotoxicosis in which all the above-mentioned values may be in the borderline range. Therefore, there is still a need for further tests in differentiating between hyperthyroidism and euthyroidism.

It has previously been demonstrated that desiccated thyroid and thyroxine administered by mouth for a week or two reduce the I^{131} uptake by the thyroid gland in normals (6, 12, 15). Triiodothyronine gives an even more marked and quicker depression of the I^{131} uptake than thyroxine (18). Perlmutter et al. (15) demonstrated that injection of thyroid-stimulating hormone (TSH) counteracts this suppression of the I^{131}

Submitted for publication September 6, 1962.

Table I Classification of the patients

Diagnosis	No. of pat.
Euthyroid patients	
Without goitre	13
Diffuse goitre	11
Nodular goitre	7
Operated thyrotoxicosis	10
Drug treated thyrotoxicosis	7
Operated non-toxic goitre	4
Thyrotoxicosis	
Untreated	19
Operated (with relapse)	5
Drug-treated (with relapse)	3

Two of the patients in this group had been treated with radioactive iodine, I[131]

Table II Distribution of initial protein-bound radioactive iodine (PBI^{131}) values

Diagnosis	No of pat.	PBI^{131} % of dose per litre <0.2	0.2–0.4	>0.4
Euthyroid patients				
Without goitre	11	8	2	1
Diffuse goitre	11	5	5	1
Nodular goitre	7	5	1	1
Operated thyrotoxicosis	10	0	1	9
Drug treated thyrotoxicosis	7	2	0	5
Operated non-toxic goitre	4	2	1	1
Thyrotoxicosis				
Untreated	19	1	9	9
Operated (with relapse)	5	0	2	3
Drug-treated (with relapse)	3	0	2	1
Total	77	23	23	31

Two of the patients in this group had been treated with radioactive iodine, I[131]

uptake in normal subjects. Therefore, the mechanism is presumably as follows: Administration of thyroid hormone inhibits the pituitary production of TSH which again leads to reduced thyroid function.

Gradually other investigators observed that thyrotoxic patients responded differently from normal subjects: their iodine uptake by the thyroid gland not being suppressed by thyroid substance, L-thyroxine or L-triiodothyronine (3, 4, 7, 8, 13, 14, 20). It was concluded that these suppression tests were applicable for differential diagnostic purposes to decide whether a goitre is toxic or non-toxic.

The object of the present study was to investigate the differential-diagnostic value of the suppression test using L-triiodothyronine (T) especially in cases where other diagnostic procedures have given inconclusive results.

Material

The material comprises 79 patients (table I): 13 euthyroid patients without goitre, 11 with diffuse non-toxic goitre, 7 with nodular non-toxic goitre, 19 with untreated thyrotoxicosis, 8 with recurrent thyrotoxicosis including 5 who had undergone subtotal thyroidectomy and 3 treated with antithyroid drugs. Seventeen were previously thyrotoxic patients rendered euthyroid, 8 had undergone subtotal thyroidectomy, 2 had been treated with radioactive I[131] and 7 were being or had been treated with antithyroid drugs. Lastly 4 were euthyroid patients previously thyroidectomized for non-toxic goitre.

The diagnosis of thyrotoxicosis was based primarily on clinical findings, in several cases after some time of follow-up, sometimes supported by observation of the effect of antithyroid medication, repeated determinations of the BMR, 4- and 24-hour uptake of I[131] by the thyroid gland, PBI^{131} in the serum, and the uptake of I[131] labelled triiodothyronine by the red cells. As is apparent from tables IV

Table III Change in 4- and 24-hour thyroid I^{131} uptake (initial uptake — final uptake) resulting from triiodothyronine (T_3) administration

Diagnosis	No. of pat.		4-hr I^{131} uptake (%) Before T	4-hr I^{131} uptake (%) After T	Average change (%)	24-hr I^{131} uptake (%) Before T	24-hr I^{131} uptake (%) After T	Average change (%)
Euthyroid patients								
Without goitre	13	Mean values	53	18	−35	69	29	−40
		Range	32–73	10–27		52–79	14–43	
Diffuse goitre	11	Mean values	59	20	−39	77	33	−44
		Range	34–84	12–31		61–92	22–56	
Nodular goitre	7	Mean values	56	36	−20	70	50	−20
		Range	34–84	11–77		48–100	21–90	
Operated thyrotoxicosis	10	Mean values	67	63	− 4	68	64	− 4
		Range	22–100	6–85		33–80	2–80	
Drug-treated thyrotoxicosis	7	Mean values	81	64	−17	76	63	−13
		Range	52–93	30–98		38–95	22–89	
Operated non-toxic goitre	4	Mean values	52	14	−38	70	30	−40
		Range	40–74	7–22		59–82	19–36	
Thyrotoxicosis								
Untreated	19	Mean values	73	75	+ 2	79	83	+ 4
		Range	40–107	34–107		51–103	47–115	
Operated (with relapse)	5	Mean values	81	87	+ 6	78	86	+ 8
		Range	59–100	76–100		67–86	61–115	
Drug-treated (with relapse)	3	Mean values	90	72	−18	90	84	− 6
		Range	87–94	65–77		86–94	79–87	

Two of the patients in this group had been treated with radioactive iodine, I^{131}

and V the majority of the patients in this group had only mild thyrotoxicosis.

Since the object was to study the value of the T suppression test in cases where one or more of the above-mentioned laboratory procedures had failed, the material included, also among the euthyroid subjects, a considerable preponderance of patients having an elevated I^{131} uptake. Likewise in respect of the PBI^{131} values there was an overlapping of euthyroid and thyrotoxic patients (table II). In particular it will be noted that 2 out of 11 normals and 5 out of 11 patients with non-toxic goitre had PBI^{131} values in the borderline area 0.2—0.4 and that 9 out of the 19 thyrotoxic patients were in the same range. Moreover 9 of the 10 euthyroid, operated, previously thyrotoxic patients and 3 of the 7 euthyroid patients whose thyrotoxicosis had been treated by antithyroid drugs had a PBI^{131} value exceeding 0.4 %, i.e. in the "thyrotoxic" range.

Methods

The 4- and 24-hour uptake of I^{131} by the thyroid gland was determined after oral administration of 10 μC of I^{131} (3).

Normal values 4 hours after the administration 15—45 %, 24 hours after the administration 30—70 % of the dosage. An increase

Table IV Metabolic rate, PBI^{127}, PBI^{131}, uptake of labelled L-triiodothyronine by erythrocytes, and the uptake in 19 patients with untreated thyrotoxicosis

No.	Sex	Age	Metabolic rate	PBI^{127} (μg%) (normal 3.5—8)	PBI^{131} % of dose per litre serum (normal <0.4)	T_3-uptake by erythrocytes (%) (normal 7—10.5)	4-hr I^{131} uptake (%) Before T	After T
1	♀	41	155—155	11.0	0.49	—	62	73
2	♀	20	149—150	—	0.37	6.6	40	34
3	♂	52	148—145	9.4	0.17	10.6	60	43
4	♀	21	150—135	—	1.40	15.0	105	39
5	♀	70	139—144	—	0.29	13.9	53	58
6	♀	45	132	—	0.56	—	85	62
7	♀	59	130—131	6.2	1.91	9.1	68	74
8	♀	27	134—125	10.7	0.39	—	72	87
9	♀	37	135—122	—	0.29	—	88	80
10	♀	38	129—122	10.3	0.44	12.5	65	87
11	♀	35	125—123	6.3	0.21	—	87	77
12	o	59	123	—	0.50	—	88	87
13	♀	40	120	12.8	0.40	11.5	65	71
14	♀	71	121—115	10.8—11.6	0.21	15.3	62	64
15	♀	25	116—120	6.5	0.36	9.5	107	107
16	♀	39	113—112	4.0	0.86	—	76	89
17	♀	17	107	10.6	3.19	11.3	97	99
18	♀	56	—	—	2.80	8.3	54	53
19	♂	63	—	8.8	0.36	12.7	50	51

Owing to difficulties in the PBI^{127} analysis at the time concerned, these values are available only for some of the patients.

or decrease > 5 % is significant (twice the standard deviation). In some cases the I^{131} uptake appears to be in excess of 100. The explanation is that the conditions relating to the geometry and "back scattering" are not entirely identical in counting the standard and in counting over the thyroid gland. At the time of the 24-hour counting, venous blood is drawn for determination of radioactive, protein-bound iodine in the serum, PBI^{131}. Normal values < 0.2 % of the dose per litre serum, questionably elevated 0.2—0.4 %, elevated > 0.4 %.

Thereupon, the patients receive L-triiodothyronine (T_3) (Tertroxin, Glaxo) 40 μg 3 times daily for six days. On the morning of the 6th day the residual quantity of I^{131} in the thyroid gland is determined, and another dose of 10 μC is administered by mouth.

The 4- and 24-hour values are read again, the residual quantity in the thyroid gland being subtracted from both uptake determinations. In calculating the 24-hour value the residual quantity is corrected to 92 % of the measured value corresponding to the physical decay of I^{131}. No regard was paid to the slight decrease due to the glandular release of I^{131} from the residual quantity, but the error on this account would be negligible.

Naturally occurring protein-bound iodine in the serum, PBI^{127}, was determined by a modification of Barker's method (2). Normal values 3.5—8 μg/100 ml.

By the method used in the present study the uptake of radioactive triiodothyronine by the red cells showed normal values from 7 to 10.5 %.

clinical evaluation compared with the L-triiodothyronine (T3) suppression of the 4- and 24-hour I^{131} thyroid

Change in 4-hr uptake (%)	24-hr I^{131} uptake (%)		Change in 24-hr uptake (%)	Comments
	Before T	After T		
+ 13	78	85	+ 7	Thyrotoxic, nodular goitre
− 8	62	54	− 8	Thyrotoxic, diffuse goitre
− 12	71	85	+ 14	Moderately thyrotoxic, no goitre
− 6	92	95	+ 3	Moderately thyrotoxic, diffuse goitre
+ 5	80	83	+ 3	Thyrotoxic, no goitre
− 3	81	76	− 5	Moderately thyrotoxic, diffuse goitre
+ 8	68	67	− 1	Doubtfully thyrotoxic, nodular goitre
+ 15	74	100	+ 26	Thyrotoxic, diffuse goitre
− 8	87	85	− 2	Slightly thyrotoxic, diffuse goitre slight exophthalmos
+ 22	80	106	+ 26	Moderately thyrotoxic, no goitre
− 10	89	85	− 4	Slightly thyrotoxic, diffuse goitre
− 1	90	95	+ 5	Slightly thyrotoxic
+ 6	87	81	− 6	Slightly thyrotoxic diffuse goitre
+ 2	66	84	+ 18	Slightly thyrotoxic. No goitre. Hypophysectomized 1958 for skeletal metastases
0	103	115	+ 12	Slightly thyrotoxic, diffuse goitre, exophthalmos
+ 15	76	90	+ 14	Thyrotoxic, diffuse goitre
+ 2	77	61	− 16	Slightly thyrotoxic, exophthalmos
− 1	51	47	− 4	Clearly thyrotoxic, diffuse goitre
+ 1	71	75	+ 4	Thyrotoxic, diffuse goitre

Determination of PBI^{131} in patient serum after the T suppression test was not performed. This might have been of some value, but as a correction has to be made for the amount of PBI^{131} already present in the serum, such a determination is complicated and can be done only with limited accuracy.

Results

Table III and figs. 1—6 give the results of the triiodothyronine suppression of the I^{131} uptake.

It may be seen from table III that the average 4- and 24-hour uptake during T suppression in euthyroid subjects without goitre decreased by 35 % and 40 % respectively in the patients with non-toxic, diffuse goitre by 39 % and 44 % respectively and in the patients with nodular non-toxic goitre by 20 % and 20 % respectively.

The thyrotoxic patients who had been subtotally thyroidectomized or treated with I^{131} and who were euthyroid at the time of the study showed an average fall of only 4 % in the 4-hour as well as 24-hour uptake. In the euthyroid patients whose thyrotoxicosis had been treated by antithyroid drugs the 4-hour and 24-hour uptakes decreased by 17 % and 13 % respectively.

Finally the 4 patients who had undergone operation for non-toxic goitre

Table IV Metabolic rate PBI[127] PBI[131] uptake of labelled L-triiodothyronine by erythrocytes, and the uptake in 19 patients with untreated thyrotoxicosis

No.	Sex	Age	Metabolic rate	PBI[127] (μg%) (normal 3.5—8)	PBI[131] % of dose per litre serum (normal <0.4)	T_3-uptake by erythrocytes (%) (normal 7—10.5)	4-hr I^{131} uptake (%)	
							Before T	After T
1	♀	41	155—155	11.0	0.49	—	62	75
2	♀	20	149—150	—	0.57	6.6	40	34
3	♂	52	148—143	9.4	0.17	10.6	60	63
4	♀	21	150—133	—	1 40	15,0	105	39
5	♀	70	139—144	—	0.29	13.9	53	58
6	♀	45	132	—	0.56	—	85	82
7	♀	39	130—131	6.2	1.91	9.1	68	74
8	♀	27	134—125	10.7	0.39	—	72	87
9	♀	37	135—122	—	0.29	—	88	90
10	♀	38	129—122	10.3	0.44	12.5	65	87
11	♀	35	125—123	6.3	0.21	—	87	77
12	♂	59	123	—	0.50	—	88	87
13	♀	40	120	12.8	0.40	11.5	63	71
14	♀	71	121—115	10.8-11.6	0.21	15.3	62	64
15	♀	25	116—120	6.5	0.36	9.5	107	107
16	♀	39	113—112	4 0	0.86	—	76	89
17	♀	17	107	10.6	3.19	11.3	97	99
18	♀	36	—	—	2.80	8.3	54	53
19	♂	63	—	8.8	0.36	12.7	50	51

Owing to difficulties in the PBI[127] analysis at the time concerned, these values are available only for some of the patients.

or decrease > 5 % is significant (twice the standard deviation) In some cases the I^{131} uptake appears to be in excess of 100 The explanation is that the conditions relating to the geometry and back scattering" are not entirely identical in counting the standard and in counting over the thyroid gland. At the time of the 24-hour counting, venous blood is drawn for determination of radioactive, protein-bound iodine in the serum, PBI[131] Normal values < 0.2 % of the dose per litre serum, questionably elevated 0.2—0.4 % elevated > 0 4 %

Thereupon, the patients receive L-triiodothyronine (T_3) (Tertroxin Glaxo) 40 μg 3 times daily for six days. On the morning of the 6th day the residual quantity of I^{131} in the thyroid gland is determined, and another dose of 10 μC is administered by mouth.

The 4- and 24-hour values are read again, the residual quantity in the thyroid gland being subtracted from both uptake determinations. In calculating the 24-hour value the residual quantity is corrected to 92 % of the measured value corresponding to the physical decay of I^{131} No regard was paid to the slight decrease due to the glandular release of I^{131} from the residual quantity but the error on this account would be negligible.

Naturally occurring protein-bound iodine in the serum, PBI[127] was determined by a modification of Barker s method (2) Normal values 3 5—8 μg/100 ml.

By the method used in the present study the uptake of radioactive triiodothyronine by the red cells showed normal values from 7 to 10.5 %

clinical evaluation compared with the L-triiodothyronine (T_3) suppression of the 4- and 24-hour I^{131} thyroid

4-hr I^{131} uptake (%)		Change in 4-hr uptake (%)	24-hr I^{131} uptake (%)		Change in 24-hr uptake (%)	Comments
Before T	After T		Before T	After T		
59	82	+ 23	78	115	+ 37	Operated upon 18 years ago. Thyrotoxic, no goitre, no exophthalmos
87	100	+ 13	96	94	− 2	Operated upon one year ago. Thyrotoxic
73	76	+ 3	79	85	+ 6	Operated upon one year ago. Slightly thyrotoxic, diffuse goitre
86	87	+ 1	72	74	+ 2	Operated upon 21 years ago. Thyrotoxic, severe exophthalmos
100	90	− 10	67	61	− 6	Operated upon one year ago. Thyrotoxic, no goitre, no exophthalmos
87	74	− 13	94	87	− 7	T_4 years treatment. Thyrotoxic, no exophthalmos
89	65	− 24	86	79	− 7	Thyrotoxic, diffuse goitre
94	77	− 17	91	85	− 6	Treated during 18 months. Doubtfully thyrotoxic, no exophthalmos

ing surgical treatment of thyrotoxicosis the 4-hour uptake increased in 4, significantly in 2, and decreased in one by 10 %. Among the three drug-treated thyrotoxic patients who were still toxic, the 4-hour uptake was suppressed in two by more than 15 % and in one by less than 15 %.

Fig. 5 shows that among 9 euthyroid, operated or I^{131}-treated previously thyrotoxic patients there was an increase in the 4-hour uptake in 2, but this was significant in only one. In 5 the uptake was unchanged or suppressed by less than 15 %, in 2 by more than 15 %.

Furthermore, it may be seen from fig. 5 that in one of the euthyroid, drug treated patients with previous thyrotoxicosis the 4-hour uptake increased. In one it decreased by less than 15 % and in the remaining 4 by more than 15 %.

Finally, all 4 patients who had undergone subtotal thyroidectomy for nontoxic goitre showed a decrease exceeding 15 %.

Discussion

The investigations confirmed the finding (3, 4, 14, 17, 20) that administration of L-triiodothyronine (T_3) suppresses the iodine uptake in the thyroid gland in normal subjects and in patients with

Table V Metabolic rate PBI¹²⁷ PBI¹³¹ uptake of labelled L-triiodothyronine by erythrocytes and the uptake in 8 patients with relapse of previously treated thyrotoxicosis

Previous treatment	No.	Sex	Age	Metabolic rate	PBI^{127} (μg %) (normal 3.5—8)	PBI^{131} % of dose per litre serum (normal < 0.4)	T_3-uptake by erythrocytes (%) (normal 7—10.5)
Subtotal thyroidectomy	20	♀	71	145	10.7	0.25	—
	21	♀	47	145—130	—	0.70	—
	22	♂	46	129	6.1	0.40	10.1
	23	♀	46	125—121	4.2	1.02	8.6
	24	♀	56	109	—	3.1	—
Antithyroid drugs	25	♀	46	174	9.9	0.26	—
	26	♀	51	122—120	—	0.24	9.4
	27	♀	66	119—110	5.7	0.73	—

Owing to difficulties in the PBI^{127} analysis at the time concerned, these values are available only for some of the patients.

showed a marked fall of 38 % and 40 % at the end of 4 and 24 hours respectively

In cases of untreated thyrotoxicosis there was an average increase in the 4-hour uptake of 2 % and in the 24-hour uptake of 4 % The previously operated thyrotoxic patients with recurrence of thyrotoxicosis also showed an increase, of 6 % and 8 % respectively In the drug treated patients with recurrence of thyrotoxicosis the 4-hour and 24-hour uptake fell by 18 % and 6 % respectively

Alterations in the 4-hour and 24-hour uptake of I^{131} following T_3 are presented in figs. 1—6

Fig 1 shows that the 4-hour uptake in all 24 euthyroid patients without goitre or with diffuse goitre was suppressed by more than 10 % In 23 of 24 patients the suppression exceeded 15 % and in 15 it exceeded 30 % On the other hand, the 4-hour uptake was suppressed by less than 15 % in 3 out of 6 patients with nodular non toxic goitre.

It is evident from fig 3 that in the 19 untreated thyrotoxic patients the 4-hour uptake was not in any case suppressed by more than 15 % In 10 it increased. In 7 (37 %) of the patients the increase was significant (> 5 %) In 4 it exceeded 10 %.

Another fact apparent from fig 3 is that in 5 patients with recurrence follow

clinical evaluation compared with the L-triiodothyronine (T_3) suppression of the 4- and 24-hour I^{131} thyroid

4-hr I^{131} uptake (%)		Change in 4-hr uptake (%)	24-hr I^{131} uptake (%)		Change in 24-hr uptake (%)	Comments
Before T	After T		Before T	After T		
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86	87	+ 1	72	74	+ 2	Operated upon 21 years ago. Thyrotoxic, severe exophthalmos
100	90	− 10	67	61	− 6	Operated upon one year ago. Thyrotoxic, no goitre, no exophthalmos
87	74	− 13	94	87	− 7	Two years' treatment. Thyrotoxic, no exophthalmos
89	65	− 24	86	79	− 7	Thyrotoxic, diffuse goitre
94	77	− 17	91	85	− 6	Treated during 18 months. Doubtfully thyrotoxic, no exophthalmos

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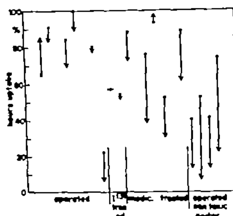


Fig. 5. Effect of L-triiodothyronine (T_3) on the 4-hour I^{131} uptake by the thyroid gland in 15 euthyroid patients treated for thyrotoxicosis as follows: in 7 cases by subtotal thyroidectomy, in 2 by I^{131}, in 6 by antithyroid drugs; also in 4 patients who had undergone operation for non-toxic goitre.
Symbols as in fig. 1

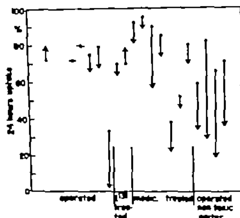


Fig. 6. Effect of L-triiodothyronine (T_3) on the 24-hour uptake by the thyroid gland in 17 euthyroid patients treated for thyrotoxicosis by subtotal thyroidectomy in 8 cases, by I^{131} in 2, and by antithyroid drugs in 7; also in 4 patients who had undergone operation for non-toxic goitre.
Symbols as in fig. 1

in 23 out of 24, i.e. 96 % of the patients. In contradistinction, the untreated thyrotoxic patients showed an average increase in the 4-hour uptake of 2 % and in no case a decrease exceeding 15 %.

Among the euthyroid patients, without or with diffuse goitre, two had elevated PBI^{131} values, > 0.4 of the dose per litre serum, and in 7 the PBI^{131} was at the upper limit of normal, 0.2—0.4 %. In 8 of these 9 patients the 4-hour uptake of I^{131} was suppressed by more than 1 %. In the 9 thyrotoxic patients whose PBI^{131} values were in the range 0.2—0.4 % the uptake was invariably suppressed by less than 15 %.

Thus, in these cases the T suppression test has yielded more information than the determination of PBI^{131}.

From tables IV and V it may be seen that a number of the thyrotoxic patients showed only slight clinical signs of toxicity (cases 7, 9, 11, 12, 13, 14, 15, 17, 22, 27) having only slightly elevated BMR. Some had normal PBI in the serum (cases 7, 11, 15, 16, 22, 23, 27) and as already mentioned only questionably elevated PBI^{131} (cases 2, 3, 5, 8, 9, 11, 14, 15, 19, 20). These cases, then, represent patients in whom, despite these investigations, there was doubt about the diagnosis and in whom the T suppression test was, therefore, of particular differential diagnostic value.

In cases of non toxic, nodular goitre the T suppression test does not appear to be particularly helpful since a suppression of the 24-hour iodine uptake by less than 15 was found in 4 out of 7 patients. This agrees with the findings of Perlmutter and Slater (14) and of Werner and Spooner (20).

Among the operated or I^{131} treated previously thyrotoxic and now euthyroid patients only 3 showed a 4-hour uptake suppressed by more than 10 % by T_3. The

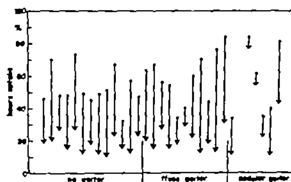


Fig. 1. Effect of L-triiodothyronine (T) on the 4-hour I^{131} uptake by the thyroid gland in 30 euthyroid patients: 13 without goitre, 11 with diffuse goitre and 6 with nodular goitre.

| before T

↓ after T

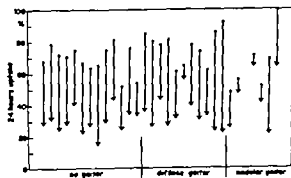


Fig. 2. Effect of L-triiodothyronine (T_3) on the 24-hour I^{131} uptake by the thyroid gland in 31 euthyroid patients: 13 without goitre, 11 with diffuse goitre and 7 with nodular goitre.
Symbols as in fig. 1.

diffuse, non-toxic goitre, but not in patients with thyrotoxicosis. The T suppression test, therefore, is of differential diagnostic value in cases where there is doubt as to whether a patient without goitre or with diffuse goitre is thyrotoxic.

It is difficult to compare the absolute values representing the T_3 suppression of the I^{131} uptake in the thyroid gland found by various authors, partly because of regional differences in respect to the I^{131} uptake and partly because the doses of T_3 have not been exactly the same.

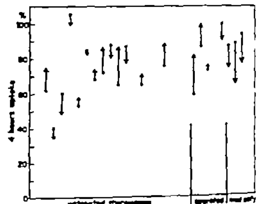


Fig. 3. Effect of L-triiodothyronine (T_3) on the 4-hour I^{131} uptake by the thyroid gland in 27 thyrotoxic patients: 19 with untreated thyrotoxicosis, 5 with recurrence following previous subtotal thyroidectomy and 3 with recurrence following drug therapy.
Symbols as in fig. 1.

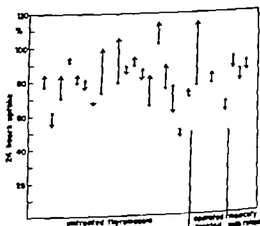


Fig. 4. Effect of L-triiodothyronine (T) on the 24-hour I^{131} uptake by the thyroid gland in 27 thyrotoxic patients: 19 with untreated thyrotoxicosis, 5 with recurrence following subtotal thyroidectomy and 3 with recurrence following drug therapy.
Symbols as in fig. 1.

Broadly speaking, however, there is agreement between the results of the above-mentioned workers and the present findings, viz. an average suppression of about 35—40 % of the 4-hour uptake in patients without goitre or with diffuse goitre and a suppression exceeding 15

capricious, there is a place for the T test from a practical diagnostic point of view Moreover t is easy to carry out, and in cases where its performance is of any interest it must be considered quite harmless.

The dosage of T used in the present study is of the same order of magnitude as that used by a number of other investigators. Apart from mild tachycardia and a tendency to sweating it did not cause any side effects.

Lastly it is worth mentioning that a number of the thyrotoxic patients showed an increase in I^{131} uptake after the administration of T_3. It should be emphasized that this was observed only in patients who were or had been thyrotoxic, never in euthyroid subjects. This paradoxical effect has also been reported by others (16, 20) It has also been observed in a thyrotoxic patient who had previously undergone total hypophysectomy (10) This peculiar phenomenon is not properly understood. The increase might be explained by assuming that the T administration does not alter the iodine uptake by the gland, but suppresses its release of organically bound I^{131} — possibly by a direct effect upon the glandular parenchyma. This problem is being investigated at present. The understanding of this entire problem complex may perhaps be facilitated by the fact that Adams (1) and McKenzie (11) demonstrated an abnormal thyroid-stimulating substance in the serum of some thyrotoxic patients. This substance L.A.T.S ("long-acting thyroid stimulator") influences the thyroid secretion over a longer period than the pituitary thyrotrophic hormone.

Summary

L-triiodothyronine (T) administered by mouth in dosage of 120 μg daily for 6 days results in a considerable suppression of the I^{131} uptake by the thyroid gland in normals. The diagnostic value of this phenomenon was investigated

The 4-hour and 24-hour uptakes of I^{131} in euthyroid patients without goitre or with diffuse goitre were suppressed by an average of 35—40 % and 39—44 % respectively and in about 95 % of the cases by more than 15 %. Patients with thyrotoxicosis, on the other hand, showed a slight increase in the I^{131} uptake after T_3. In about 40 % of the patients the increase was significant (> 5 %) and no case showed a decrease exceeding 15 %.

Where the ordinary thyroid tests fail the T suppression test is, consequently an excellent means of deciding whether or not patients without goitre or with diffuse goitre are suffering from thyrotoxicosis. If the I^{131} uptake is suppressed by more than 15 % the patient is probably not hyperthyroid

Among patients with nodular nontoxic goitre T suppressed the I^{131} uptake in about half the cases by less than 15 %. Thus, a negative result of the test in patients with nodular goitre does not rule out that the goitre may be non-toxic.

Patients who have undergone subtotal thyroidectomy for thyrotoxicosis and who have a recurrence respond to the T suppression test like untreated patients with thyrotoxicosis, some showing an increase and none a decrease of more than 15 %

Patients who have been rendered euthyroid by subtotal thyroidectomy or by treatment with I^{131} on the other hand respond in a different way from untreated euthyroid patients, the I^{131} uptake in only a few of them decreasing by more than 15 % after T and in some even increasing This happens regardless of whether or not exophthalmos is present.

operations had been performed 20 years, 3 years, and 5 months ago. Two had mild exophthalmos. In the remaining 7 euthyroid previously thyrotoxic, patients in this group the 4-hour uptake was suppressed by less than 10 % by T_3. Four of them had been treated by operation — or by I^{131} — less than one year before the investigation and 3 from one to 12 years previously. Of these 7 patients 3 had mild to moderate exophthalmos while 4 had normal eye findings. These observations, then, merit the conclusion that the absence of T_3 suppression in a patient operated upon for thyrotoxicosis is not tantamount to his being thyrotoxic, regardless of whether exophthalmos is present or not. This agrees with the investigations of Werner (19) who followed patients thyroidectomized or I^{131} treated for thyrotoxicosis by the T_3 suppression test, and found that in the majority the iodine uptake was not suppressed by T_3 within the first 5 years. In a few cases, it was not even suppressed 20 year after the treatment, even if the patients were euthyroid. Morgans et al. (12), Werner and Spooner (20) and Hales et al. (9) on the other hand found a good correlation between the degree of T_3 suppression and the patient's ultimate clinical status.

That the lack of suppression in the patients operated upon for thyrotoxicosis is related to their previous thyrotoxicosis, and is not merely a consequence of the thyroidectomy is supported by the fact that in all 4 patients who had undergone subtotal thyroidectomy for non toxic goitre the iodine uptake was suppressed by T_3.

In respect to the thyrotoxic patients who had been treated with antithyroid drugs, usually 1 methyl 2 mercaptoimidazole, matters are complicated as some of them had received antithyroid medication during or up to the T_3 suppression while others had been off the drug for a long time before the test. Continued investigations may perhaps show whether the T_3 suppression can afford guidance as to when it is permissible to discontinue antithyroid medication.

As to whether the 4-hour or 24-hour I^{131} uptake in the T_3 suppression test is to be preferred it must be said that the two determinations appear to be of approximately equal differential diagnostic value. The 24-hour determination (table III figs. 2 4 and 6) distinguishes just as well as the 4-hour test between untreated euthyroid patients and patients with thyrotoxicosis. On the other hand, both fail in many cases of euthyroid patients treated surgically for thyrotoxicosis. That in a number of cases there is a discrepancy between the two determinations may be explained by the fact that the 4-hour and 24-hour values represent merely two points on the curve of the I^{131} turnover in the thyroid gland. It would be more accurate to determine the "I^{131} uptake rate" (8) but this was not done in the present study which was designed to investigate the practical, clinical value of the T_3 suppression test.

From what has been stated above, it is apparent that the T_3 suppression test may be of value especially where the diagnosis of hyperthyroidism is in doubt and where the other tests have shown results in the borderline range. Regrettably it affords no help in deciding the frequently very difficult problem as to whether there is a mild recurrence in a previously thyrotoxic patient treated with subtotal thyroidectomy or I^{131}.

Strictly speaking therefore the T_3 is rather seldom called for. Since, however it is well-known that a number of the other tests (BMR and PBI [17]) may be

capricious, there is a place for the T test from a practical diagnostic point of view Moreover t is easy to carry out, and in cases where its performance is of any interest it must be considered quite harmless.

The dosage of T used in the present study is of the same order of magnitude as that used by a number of other investigators. Apart from mild tachycardia and a tendency to sweating it did not cause any side effects.

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Therefore, the T_3 suppression test is of little differential diagnostic value in patients of this category

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From the Institute of Hygiene, the University Oslo, Norway

Studies on Hemoglobin Values in Norway

I. Hemoglobin Levels in Adults

By

Haakon Natvig

A voluntary industrial health service program was introduced in Norway in 1943 (28) and has since been adopted by about 1,200 industries and business companies with altogether some 250 000 employees. A part of this program is an annual or biannual physical examination of all employees. The physical examinations are standardized to the extent that the physicians working in the health program are required to make certain specified examinations and clinical tests and to record their findings on a standard health record form. This degree of uniformity makes the results of the physical examinations, when collected at a central office, a valuable source of information about health conditions of a fairly large proportion of the adult population. The board of the industrial health service therefore asked the physicians to report to the board, on a standard form, some of the results of all the physical examinations performed during the calendar year 1952

This material has been used in a study of the height-weight relationship of adults (25) and in an analysis of the correlation between blood pressure and body weight (3). To be presented in this report is an analysis of all the hemoglobin values reported. Hemoglobin values are analysed with regard to sex, age marital status, occupation, and place of employment. Although the analysis is basically descriptive, findings would seem to support the view that iron deficiency anemia is more frequent in certain age groups than previously thought, and they indicate that it would be reasonable to recommend a supplementary iron supply as a prophylactic measure, especially for adult men below 20 and above 50 years of age and for adult women from maturity until menopause.

Material and methods

Hemoglobin values were reported for altogether 23,901 persons, 17,358 men and 6,543 women, working at 112 companies located in the southern parts of Norway. Table I shows the age distribution of the persons examined. This material is not a fully representative sample of the adult population of

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Table I Sex and age distribution of the persons examined

Age (yrs)	Men			Women		
	Wage earners	Salaried employees	Total	Wage-earners	Salaried employees	Total
15–19	904	134	1 038	811	177	988
20–29	2,762	837	3,599	1,326	1,103	2,429
30–39	3,288	1,264	4,552	735	464	1,199
40–49	2,747	954	3 701	665	343	1,008
50–59	2,066	865	2,931	456	276	732
60 and > 60	1 112	409	1,521	122	62	184
Age unknown	14	2	16	1	2	3
Total	12,893	4 465	17,358	4 116	2,427	6,543

Table II The companies and persons grouped according to occupations

Occupation	Companies	Persons
Iron, basic metal & mechanical industries	28	5 114
Mining & quarrying industries	14	5,399
Textile industries	14	3 723
Chemical industries	13	2,891
Food industries	13	1 417
Wood-pulp & paper industries	6	1 014
Commerce, transport, banks & insurance companies	24	4,343
Total	112	23,901

Table III The companies and persons grouped according to place of employment

District	Companies	Persons
Oslo	34	5,526
Eastern Norway excl. Oslo	53	9,267
Southern Norway	3	2,025
Western Norway	17	6 445
South Trondelag	5	638
Total	112	23,901

Norway but represents an unselected sample of men and women able to take on jobs as laborers and office workers. Traditionally official statistics in Norway deal with manual laborers, persons paid by the hour whom for the sake of convenience we may term "wage earners" and office staff, persons with clerical supervisory or managerial posts paid by the month, whom we may call "salaried employees"

The once marked differences in the standard of living between "wage-earners and "salaried employees" have diminished considerably in the last decades. Nowadays, many wage-earners have an easy and sedentary occupation indoors, hardly distinguishable from the mechanical work done by many of the salaried employees. On the other hand, many of the salaried employees work in the factories as foremen and technicians. Even though there is no sharp distinction in social or hygienic conditions, wage-earners have generally greater physical activity and more outdoor work than salaried employees and are to a greater extent exposed to dust, toxic substances and other health hazards. It will therefore be of interest to investigate the hemoglobin values of the two groups separately. The number of persons in each group is shown in table I

Other groupings of the material used in the analysis of hemoglobin values are shown in tables II and III. In table II the material is grouped according to broad occupational groups, and in table III according to place of employment.

Blood samples have been withdrawn on working days of the year between 8 a.m. and 4 p.m. from persons sitting upright. Many factors known to affect the hemoglobin concentration of the blood are therefore not controlled. That such factors, e.g. temperature, physical activity, time of the day, season, etc. should introduce any systematic error in any of the comparisons made in the present study is highly unlikely. On the other hand, and for the same reason, the actual hemoglobin values in the present material are not directly comparable with values obtained under strict standardized conditions.

Well-trained registered nurses sampled the blood and performed the hemometer readings. Differences between nurses in blood sampling technique and reading of course occur, and such differences should be taken into consideration in the comparison of records from one particular company with records from another company. The groupings of the material on which the analysis of hemoglobin values is based, however, will always include records from a number of companies, i.e. readings by a number of different nurses, and it is therefore unlikely that reading differences should invalidate the comparisons made.

The hemoglobin values have been determined colorimetrically by the Sicca method, using venous blood in all cases. Three to five drops of blood are sucked into a miniature pipette and at once hemolysed and reduced by adding a small amount of Sicca powder. In the Sicca hemometer (Testa Laboratorium, Copenhagen) which is easily operated, a layer of constant thickness of the reduced hemoglobin is compared with a standard color using a constant source of light. The accuracy of the Sicca method is reported to be within ± 3 % in the hands of an experienced person (20, 34, 36).

All the Sicca hemometers in routine use in *the industrial health program are standardized* according to the Haldane standard, i.e. 18.5 vol. % O_2 = 13.8 g hemoglobin per 100 ml blood = 100 %, and based on an extinction coefficient of 10.8.

Table II. Distribution of the hemoglobin values for all men and women (100 % = 13.8 g Hb/100 ml blood)

Hb %	Men		Women	
	No.	Percentage	No.	Percentage
67 and below	9	0.05	21	0.32
68–72	12	0.07	43	0.66
73–77	12	0.07	112	1.71
78–82	63	0.36	333	5.09
83–87	138	0.80	688	10.52
88–92	748	4.31	1 796	27.45
93–97	1,510	8.70	1,685	25.75
98–102	4,010	23.10	1,282	19.59
103–107	4,137	23.83	419	6.40
108–112	4,199	24.19	129	1.97
113–117	1 643	9.47	30	0.46
118–122	702	4.04	5	0.08
123–127	14	0.08	—	—
128 and above	28	0.12	—	—
Total	17,338	100.00	6,543	100.00

Table V. Mean hemoglobin values in the different age groups (100 % = 13.8 g Hb/100 ml blood)

Age (yrs)	Men			Women		
	No.	Mean Hb	S. E.	No.	Mean Hb %	S. E.
15–19	1,058	102.6	± 0.27	988	93.0	± 0.25
20–29	3,399	108.4	± 0.12	2,429	93.3	± 0.16
30–39	4,332	106.0	± 0.11	1,195	92.8	± 0.23
40–49	3,701	105.0	± 0.12	1,008	93.1	± 0.27
50–59	2,931	104.0	± 1.15	732	94.2	± 0.29
60–69	1,501	102.4	± 0.27	184	93.4	± 0.61
Total	17,312	105.0	± 0.06	6,540	93.2	± 0.09

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128 and above	28	0.12	—	—
Total	17,358	100.00	6,543	100.00

Table I Mean hemoglobin values in the different age groups (100 % = 13.8 g Hb/100 ml blood)

Age (yrs)	Men			Women		
	No.	Mean Hb %	S. E.	No.	Mean Hb %	S. E.
15–19	1,038	102.6	± 0.27	968	93.0	± 0.25
20–29	3,599	106.4	± 0.12	2,425	93.3	± 0.16
30–39	4,552	106.0	± 0.11	1,399	92.8	± 0.25
40–49	3,701	105.0	± 0.12	1,008	93.1	± 0.27
50 59	2,831	104.0	± 1 15	732	94.2	± 0.29
60–69	1,321	102.4	± 0.27	164	93.4	± 0.61
Total	17,342	105.0	± 0.06	6,540	93.2	± 0.09

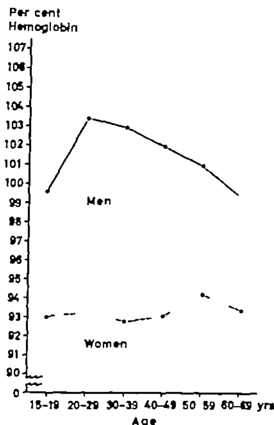


Fig 1 Mean hemoglobin values by age.

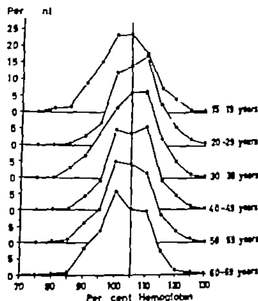


Fig 2. The relative distribution of the hemoglobin values in age groups in males.

Results

1 *Distributions and mean hemoglobin values in men and women*

Table IV shows the distribution of the hemoglobin values at 5 % intervals for all men and women From table V it appears that the mean value for all men is 105 % (S D = 7 4 S E. = 0 06) and for all women 93 2 % (S D = 7 8, S E = 0 1) These values correspond to 14 5 and 12 9 g hemoglobin per 100 ml blood respectively

The hemoglobin values, however differ according to age (cf table V) The values are low in men of 15—19 years, reach a peak at 20—29 years, and slowly decline to a new low value in elderly men It is of interest to note that the hemoglobin values in women increase after the age of 50 The differences between the age groups in men are statistically significant. For testing the significance between the means of two groups the t-test is used

$$t = \frac{m - m_2}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}}$$

If t is more than ± 2.5 the difference is considered statistically significant. In women the differences are not significant except between the age group 40—49 and 50—59 The mean hemoglobin values according to age in men and women are illustrated in fig 1

The frequency distributions of the hemoglobin values in the various age groups are shown in fig 2 for men and in fig 3 for women The mean hemoglobin value for all age groups is marked by the vertical line. The distributions in men are fairly symmetrical. In the age groups 20—29 and 30—39 the curves shift to slightly higher values, while the distributions in the older age groups seem to indicate an excess of values slightly lower

Table VI The frequency of low hemoglobin values in the various age groups

Age (yrs)	% of men with Hb values below		% of women with Hb values below	
	90% or 12.5 g	85% or 11.8 g	80% or 11.0 g	75% or 10.4 g
15—19	2.6	1.2	2.3	0.5
20—29	0.4	0.1	2.6	0.7
30—39	0.8	0.2	3.5	1.8
40—49	1.0	0.5	3.5	1.0
50—59	1.7	0.7	1.0	0.5
60—69	3.5	1.5	1.6	0.5
Total	1.4	0.6	2.7	1.0

than average In women, such an excess of values slightly lower than average age is marked in all ge groups except 15—19 and 50—59 years.

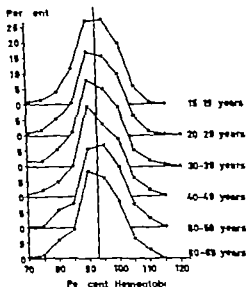


Fig. 3. The relative distribution of the hemoglobin values in age groups in females.

2. *The frequency of low hemoglobin values in the various age groups*

Of particular interest from a clinical and prophylactic point of view is the frequency with which low hemoglobin alues are observed. The frequencies with which values below 90 and 85 % occur in men and values below 80 and 75 % occur n women appear in table VI The age trend for men and women with regard to frequency of low hemoglobin values is strikingly different, as can be seen from fig 4. About the same frequency of low values for men and women is observed for the age group 15—19 In men the percentage of low values rapidly declines to a minimum in the age group 20—29 then t increases progressively with increasing age In women percentage of low hemoglobin values increases up to the age of 50 then an abrupt decline occurs followed by slight increase in women between the ages of 60 nd 69 years.

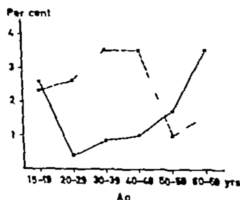


Fig 4. Percentage of low hemoglobin values by age.
— — Men below 90 % hemoglobin.
— Women below 80 % hemoglobin.

3 *Hemoglobin values in wage-earners and salaried employees*

The mean hemoglobin value in male wage-earners of all ages combined is 104.8 % as compared with 105 4 in male salaried employees. This difference is

Table VII Hemoglobin values for male wage-earners and salaried employees by age

Age (yrs)	Wage-earners			Salaried employees		
	No.	Mean Hb %	S. E.	No.	Mean Hb %	S. E.
15—19	904	102.4	± 0.28	134	103.9	± 0.68
20—29	2,762	106.1	± 0.14	837	107.4	± 0.36
30—39	3,288	106.0	± 0.13	1,264	106.1	± 0.[illegible]1
40—49	2,747	104.9	± 0.15	954	105.1	± 0.23
50—59	2,066	103.8	± 0.17	865	104.3	± 0.27
60—69	1 112	102.0	± 0.25	409	103.5	± 1.32
Total	12,879	104.8	± 0.07	4 463	105.4	± 0.12

Table VIII Hemoglobin values for female wage-earners and salaried employees by age

Age (yrs)	Wage-earners			Salaried employees		
	No.	Mean Hb %	S. E.	No.	Mean Hb %	S. E.
15—19	811	92.9	± 0.25	177	93.8	± 0.60
20—29	1,326	93.2	± 0.20	1,103	93.3	± 0.23
30—39	735	93.0	± 0.31	464	92.5	± 0.34
40—49	665	93.5	± 0.29	343	93.0	± 0.45
50—59	456	94.6	± 0.35	276	94.6	± 0.47
60—69	122	92.5	± 0.69	62	95.2	± 0.89
Total	4 115	93.2	± 0.12	2,425	93.4	± 0.16

Table IX Hemoglobin values in married and single male wage-earners

Age (yrs)	Married			Single		
	No.	Mean Hb %	S. E.	No.	Mean Hb %	S. E.
15—19	(6	101.5	± 3.03)	897	102.2	± 0.29
20—29	990	105.8	± 0.24	1 743	106.2	± 0.18
30—39	2 460	105.8	± 0.16	768	105.8	± 0.27
40—49	2,410	104.8	± 0.16	324	104.6	± 0.43
50—59	1 782	103.8	± 0.19	272	103.2	± 0.41
60—69	939	101.8	± 0.27	162	101.0	± 0.60
Total	8,587	104.8	± 0.1	4 166	104.8	± 0.13

statistically significant (t = 4.5) and it is not caused by different age distribution, as appears from table VII The differences between wage-earners and salaried employees are, except for age groups 30—39 and 40—49 statistically significant. Corresponding data for female workers and employees are presented in table VIII

Table X. Hemoglobin values in married and single women

Age (yrs)	Married			Single		
	No.	Mean Hb %	S. E.	No.	Mean Hb %	S. E.
15—19	(15	93.6	± 2.1)	873	93.1	± 0.26
20—29	588	92.9	± 0.41	2,032	93.3	± 0.19
30—39	437	93.0	± 0.57	741	92.5	± 0.29
40—49	461	93.1	± 0.37	544	93.0	± 0.33
50—59	258	94.7	± 0.53	472	94.0	± 0.37
60—69	(49	94.3	± 1.4)	134	93.1	± 0.68
Total	1,629	93.3	± 0.21	4,896	93.2	± 0.12

Table XI. Hemoglobin values of wage-earners in various occupations

Type of industry	Male wage-earners			Female wage-earners		
	No.	Mean Hb value	S. E.	No.	Mean Hb value	S. E.
Iron, basic metal and mechanical	3,332	106.8	± 0.14	579	94.8	± 0.33
Textile	1,257	105.3	± 0.22	2,025	94.3	± 0.17
Chemical	1,453	105.2	± 0.20	443	90.5	± 0.35
Commerce, transport etc.	1,015	104.3	± 0.27	260	92.6	± 0.46
Mining and quarrying	4,294	102.9	± 0.12	314	94.3	± 0.40
Food	688	102.3	± 0.32	420	93.4	± 0.36
Wood-pulp & paper	809	101.9	± 0.23	(72	93.8	± 0.77)

The differences are small and in no case are they statistically significant.

4. *Hemoglobin values according to marital status*

As a difference in hemoglobin values between male wage-earners and salaried employees has been demonstrated, the analysis of hemoglobin values according to marital status in men is based on male wage-earners only. The result is presented in table IX. The mean hemoglobin value is 104.8 in both married and single. However the hemoglobin values in single male wage-earners, including divorced as well as widowers, are somewhat higher in men below 50 and slightly lower in men above 50 years of age. The differences in the various age groups, however, are small and not significant. Table X indicates that similar small, but not significant differences occur also between married and single women. The comparison has been based on all women since no significant differences occurred in the mean hemoglobin values between female wage-earners and salaried employees.

5. *Hemoglobin values of wage-earners in various occupations*

The mean hemoglobin values for male and female wage-earners in different occupational groups are presented in table

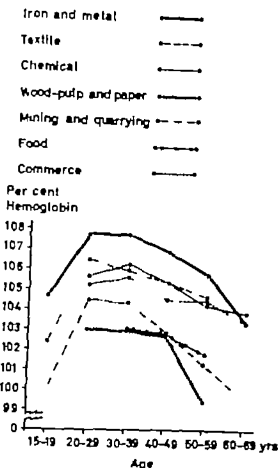


Fig 5 Hemoglobin values of male wage-earners in various occupations.

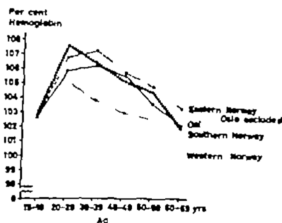


Fig 6. Mean hemoglobin values in various districts.

XI The values are highest in male wage-earners in the iron basic metal and mechanical industries (the "iron group") followed by the textile and chemical industries and the commerce, bank insurance and transport companies (the "commerce group") The mean hemoglobin value in wage-earners in the iron group is significantly higher than the values observed in the other occupational groups. The small differences between the textile, chemical and commerce groups are statistically not significant. On the other hand significant differences occur between these groups and the food, the mining and quarrying and the wood-pulp and paper industries, which have the lowest mean value. Similar differences are found in all age groups, as can be seen from fig 5

The highest mean hemoglobin value is also observed in female wage-earners in the iron group and it is significantly higher than that in the food, chemical and commerce groups, but it is not significantly different from the value found in the other occupational groups.

Table XII Hemoglobin values of male wage-earners in different districts

Districts	No.	Mean Hb %	S.E.
Oslo	1,849	105.0	0.23
Eastern Norway excl. Oslo	5,746	103.8	0.11
Southern Norway	1,698	105.3	0.17
Western Norway	3,268	103.4	0.12
South Trondelag	298	104.7	0.56

6 *Hemoglobin values of male wage-earners in various districts*

When grouped according to place of employment (cf table III) the mean hemoglobin values of male wage-earners of all ages are somewhat lower in the western than in the other districts of the

country as shown in table XII This statistically significant difference occurs in all age groups (fig 6)

Discussion

Caution must be exercised when comparing different surveys of hemoglobin values because of the variety of methods used. Actual values as observed in the present survey are only comparable with values reported from other surveys after adjustments are made for methods and hemometers have been standardized in the same way

I Wintrobe (38) and others give as normal values of the hemoglobin in adult men 16 ± 2 g/100 ml blood for men and in adult women 14 ± 2 g/100 ml, which correspond to 116 % ± 14 and 101 % ± 14 respectively In Norway various authors report mean hemoglobin values in young men to be 15.5—16.2 g and in young women 13.6—14 0 g (18, 26) In a comprehensive Danish study Bierring (2) found an average hemoglobin value in men of 15.6 and in women 13 7 g In order to obtain round figures, Bierring suggests that normal values should be regarded as 110 ± 15 for men and 100 % ± 15 for women This corresponds to 15.2 g for men and 13.8 g for women.

The mean hemoglobin value for men in the present material was 105 % = 14.5 g and for women 93.2 % = 12.9 g

The most likely reason for these lower than "normal" values is that our material consists of individuals of all ages fit for work, but not of a selected sample of healthy young adults from which the "normal" figures are derived. The figures in the present material, however correspond well with those found in the majority of investigations of non-selected population groups (1 6 11 21 23 29 31) The survey of unselected adults in New South Wales however revealed higher mean values (37)

What should be regarded as "normal" hemoglobin values is in part a philosophical question. If "normal" implies hemoglobin values associated with excellent health, then norms should be based on observations of selected entirely healthy individuals. If, on the other hand, "normal" is used to mean the most frequent, norms should be based on studies of large representative samples of the population.

If the latter definition of "normal" is adopted present material would indicate that about 95 % (± 2 0 S. D from the mean) of men will have hemoglobin values between 90 and 120 % (12.5—16.5 g/100 ml) and 95 % of the women will have values between 80 and 110 % (11.0—15.0 g/100 ml) Values below 90 % (12.5 g) in men and below 80 % (11 0 g) in women would accordingly indicate anemia.

In the material here presented, a slight, but steady decline in mean hemoglobin values occurs in men beyond 30 years of age, invariably and irrespective of the group. Hawkins et al. (12) observed the same decline after the age of 30 and a similar age pattern is found in the material of Andersen and Normann (11) and that of the British Medical Research Council (6) and of *Walsh et al.* (37) with the only difference that in the last named materials the decline is first apparent from the age of 40.

There is reason to believe that many physiological factors, e.g a reduced production of testosterone (13) contribute to the decline in hemoglobin values with increasing age in men above 30 A number of factors, such as low intake of iron, a variety of diseases involving loss of blood

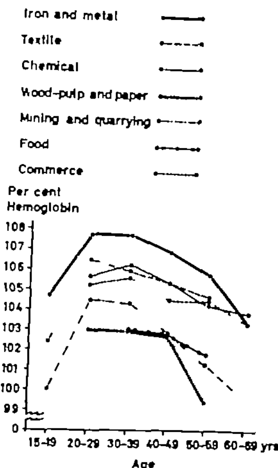


Fig 5 Hemoglobin values of male wage-earners in various occupations.

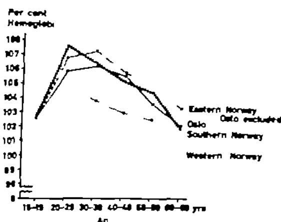


Fig 6. Mean hemoglobin values in various districts.

XI The values are highest in male wage-earners in the iron basic metal and mechanical industries (the iron group")

Table XII Hemoglobin values of male wage-ear
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Districts	No.	Mean Hb %	S.
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Southern Norway	1 698	105.3	0.1
Western Norway	3,288	103.4	0.1
South Trondelag	298	104.7	0.5

followed by the textile and chemical i dustries and the commerce, bank insu ance and transport companies (t "commerce group") The mean hem globin value in wage-earners in the ir group is significantly higher than th values observed in the other occupation groups. The small differences between th textile, chemical and commerce grou are statistically not significant. On th other hand significant differences occu between these groups and the food th mining and quarrying and the woo pulp and paper industries, which hav the lowest mean value. Similar difference are found in all age groups, as can b seen from fig 5

The highest mean hemoglobin value i also observed in female wage-earners i the iron group, and it is significantl higher than that in the food, chemica and commerce groups, but it is not signif icantly different from the value found i the other occupational groups

6 *Hemoglobin values of male wage-earners in various districts*

When grouped according to place of employment (cf. table III) the mean hemoglobin values of male wage-earners of all ages are somewhat lower in the western than in the other districts of the

Ogrum and Homb (39) in a recent study observed that the diet of industrial wage-earners did not on the average contain less iron than that of the salaried employees on the contrary the in general higher food intake by wage-earners should automatically entail a good iron supply On the other hand, families with the lowest income and families with 3 or more children did not get sufficient iron in their diet to cover the recommended daily allowance. Thus, it cannot be excluded that too low a supply of iron may be a contributing cause to the on the average, slightly lower hemoglobin values in wage-earners as compared with salaried employees.

4 Hobson and Blackburn (17) found that the elderly males living alone have lower hemoglobin values than those living with their wives. In our material no statistically significant differences could be revealed between the hemoglobin values of married and single men. A tendency towards low values, however occurred among the single in the older age groups.

The observation of somewhat lower hemoglobin values with increasing age in bachelors, divorced men, and widowers, may suggest that malnutrition may also be one factor responsible for the declining hemoglobin values found in old age.

5 Differences between mean hemoglobin values in various occupational groups may be caused by many factors, such as standards of living, exposure to toxic agents, manual labour and sweating In the material here presented the highest hemoglobin values occurred in persons i constant contact with iron such as w ge-earners in the iron, basic metal and mechanical industries. In contrast, the lowest hemoglobin values have been observed in wage-earners in such industries as the wood-pulp and paper and the food industries, in which there probably is not such contact with iron.

6 Recent reports by physicians in the industrial health service seem to confirm that a difference in the hemoglobin values of adults exists in various parts of the country The low values reported from northern Norway (10 19 33) are striking, and contrast markedly with the findings of the present investigation.

In this connection it is of particular interest to note that food consumption surveys (39) indicate that the average iron content of the diet is about the same in families of industrial workers in the various parts of the country However ascorbic acid has been found to enhance absorption of iron (4 5 16, 35) and it is well known that vitamin-C supplies in the northernmost part of Norway may be low for several months of the year It is possible therefore that the low hemoglobin values reported from northern Norway are related to insufficient vitamin-C supplies.

Summary

The analysis of the material here presented has revealed relatively low mean hemoglobin values and a high frequency of anemia in men between 15 and 19 and above 50 years of age and in women from maturity until menopause. Recent food consumption surveys indicate that the iron content of Norwegian diets is about 8 mg per 2,000 calories. The low hemoglobin values observed may be caused by insufficient intake of iron. It would seem reasonable therefore to recommend a supplementary iron intake for certain groups of the adult population in Norway

or reduced absorption of iron, chronic infections, etc. may be contributory The studies of Orchard (30) and Gillum et al. (9) who did not find any decline in the hemoglobin values up to 70 years of age for elderly and old men in excellent health and nutritional state and with a sufficient iron intake support such a view

The relatively low mean hemoglobin value in men 15—19 years may in part be due to the fact that some of these young men may not have reached full maturity However it may also indicate primary iron-deficiency anemia (7)

The age pattern of hemoglobin values in women a slight and not significant increase from the age group below 20 years to the age group 20—39 years, is probably due to the fact that unlike men women at the age of 15—19 years have already reached full physical maturity The most characteristic feature of the age pattern for women however is the relatively low hemoglobin values for those between 30 and 50 years of age and the high value in the age group 50—59 An almost identical age pattern was found by Walsh et al. (37) in New South Wales. This pattern no doubt is related to the child bearing period and its cessation In this period women have an iron requirement of 1—1 5 mg per day and during pregnancy 3—4 mg as against 0 5—1 mg after the menopause.

2 Low hemoglobin values are relatively frequent in young men below 20 years and in men above 50 years of age. Hemoglobin values below 90 % i.e anemia were found in 2 6 % of men 15—19 years and in 3 5 % of men 60—69 years of age. Brumfitt (7) found hemoglobin values below 11 8 g unassociated with organic disease in 1 1 % of 2,000 recruits below 20 years of age Among 1 000 trained soldiers above 20 years of age however the incidence of anemia was only 0 1 % Brumfitt's theory that some of the young men develop anemia owing to an insufficient iron intake seems logical, since the iron requirement in male subjects 15 to 19 years old is so much higher than before and after this age (14 15) In our material hemoglobin values below 11 8 g occurred in 1.2 % in men 15—19 years, and in 0 1 % in men 20—29 years. Thus primary iron-deficiency anemia may occur among Norwegian young men with about the same frequency as found by Brumfitt.

Recent investigations (22, 40) in Norway indicate that the diet of elderly persons is insufficient with regard to iron. It seems therefore a reasonable assumption that the relatively low hemoglobin values observed in elderly people are connected with an insufficient iron supply This would be in accordance with the investigations of Lange and Skjeggestad (24) and of Qvigstad (32) who have shown that iron deficiency anemias increase with increasing age.

In women contrary to what is found in men the highest frequency of low hemoglobin values occurs in the age between 20 and 49 years. Evidence points to an insufficient iron intake as the main cause.

3. The slightly lower hemoglobin values in wage-earners as compared with salaried employees, may in part be explained by greater morbidity of the wage-earners, and/or by the fact that the wage-earners are exposed to toxic substances to a greater extent. Other environmental factors may influence the hemoglobin value for instance iron loss in sweat (8, 27) particularly in wage-earners with heavy manual labour in hot environments.

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From the Third Medical Department, University of Helsinki, Helsinki, Finland

The Milk-alkali Syndrome

A Report of three Illustrative Cases and a Review of the Literature

By

Sven Punsar and Timo Somer

Sippy in 1912 introduced the milk and alkali treatment for peptic ulcer Early in the century there appeared only a few reports on the hazardous effects caused by an excessive application of the regime (a.o 18) Hardt and Rivers in 1923 gave a detailed account of the toxic mani festations following the alkaline treat ment of peptic ulcer (20) They found in their cases symptoms and signs of acute alkalosis and temporary renal insuffic iency which promptly disappeared after discontinuance of the therapy This syndrome has since been reported frequently (6, 17 22, 25)

Subsequently Cope in 1936 pictured a wider spectrum of the complications from the alkaline treatment of peptic ulcer in describing in four patients a syndrome of marked hypercalcemia hyperphosphatemia, azotemia and elevated plasma bicarbonate with decreased serum chloride (7) The chemical abnormalities and the resulting subjective symptoms quickly subsided following the discontinuance of antacid therapy although the signs of impaired renal function persisted for several weeks after subjective recovery

Submitted for publication September 18, 1962.

While observations on Cope s syndrome were occasionally reported t was not until 1949 that Burnett et al. renewed the interest on the injurious effects of this kind of therapy (3) They described in six patients, each with a chronic duodenal ulcer and a history of ingestion of large quantities of milk and absorbable alkali, a syndrome which closely simulated primary hyperparathyroidism with secondary renal damage. The main signs were hypercalcemia without hypercalciuria or hypophosphatemia, a normal serum alkaline phosphatase, marked chronic renal insufficiency mild alkalosis, and metastatic calcinosis of various tissues. The renal insufficiency of these patients had a gra e prognosis, only two of the six patients showing some clinical improvement following the restriction of the intake of milk and absorbable alkali.

It has not often been noted that the syndromes described above actually represent different stages of the same disease, the milk-alkali syndrome (38, 48) Apparently cases with symptoms described by Hardt and Rivers belong to the first phase of the disease, while those

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Table I. Laboratory data in cases nos. 2 and 3

		Blood										Urine					
		Ca (mg %)	P (mg %)	Alkaline phosphatase B. L. U.	NPN (mg %)	Creatinine (mg %)	Bicarbonate (mEq/l)	Na (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Hb (g %)	Specific gravity	pH	Protein	Ca (mg/24 hr)	P (mg/24 hr)	Pyuria
Case 2																	
1961																	
June	15	11.6	3.6	1.4	62	4.9	30	135	3.3	89	12.3	1,011	6.7	–			±
	19	12.7	3.2	1.6	54	4.1		137	3.1	92		1,008		–	26	700	–
	21	9.6	2.7	1.2		3.3	29		3.6	109	11.5		7.0		61	200	
	26	12.5	3.4	1.0	26	2.6	22	146	4.4	108	10.2	1,014			105	900	
Oper.	28					2.9		139	4.1			1,016			45	100	
	30	13.0	4.4		26	2.7	24	137	3.6	98	13.3	1,010					
July	1	13.2	4.8			2.6	23	141	4.4	104	12.4	1,015					
	3	9.7				2.3	21	144	4.4	101			5.4		80	200	
	6	9.3	4.3	2.0	26	1.8	21	137	4.0	102	11.8	1,010	5.6		67	390	
Ambul.	26	9.5				2.3			4.5		10.5						
Case 3																	
1961																	
I adm.																	
March	9	15.8	2.8		105		15	143	3.5	104	6.8	1,008	<7.0	–			–
	13	13.0			46		32	143	3.7		7.4		>7.0	–	179		+
	21	12.8	3.6	3.1	39						10.3	1,009	6.3		148		
	27	9.6	3.5	3.0	54	4.0	25						7.0	+	97		–
April	5	9.6	4.8	2.4	32			143	4.7	104	9.2	1,009	7.2	+	129		±
	8							143			9.2				167		
	14	10.6	3.9	2.1	49	3.9	21	134	3.7	102					165		
	20	10.9	4.7		33		18	142	4.6	105	11.5	1,011	5.5	±	310	700	–
	23	11.3	5.9		42	3.3	15	149	4.1				5.4				
	28	14.3	3.5	2.8	39	3.1	19	141	3.9				5.6		248	300	
	29	13.8															
May	2	11.7	2.8		31	2.9	26						6.3		285	400	
	3	11.3	2.8	1.8				143	4.2		8.7		<7.0	±	225	300	–
II adm.																	
July	20	10.5	3.9	3.1	38	3.8	19	139	4.4	104	11.7	1 005	5.7	–	104	400	–
	28	10.6	3.6			2.7	25	146	4.7	104							
	31	10.9	3.3	3.5		2.8	26	144	4.2								
Aug.	1	10.7	3.4										5.8		236	500	
1962																	
III adm.																	
March	8	9.2	3.0	1.9		2.4	26	135	5.3	112	9.8	1,006	<7.0	±			–

corresponding to Cope's cases represent the second phase the gravest cases which show the manifestations of Burnett's syndrome forming the usually irreversible stage of the disease.

These circumstances are illustrated by the following three cases, each of which have manifestations of a different phase of this disorder and two of them also exhibiting some special features of interest In addition a review is made of the literature on the two more advanced stages of the milk alkali syndrome.

Case reports

Case 1 A 38-year-old foundryman was admitted to the Third Medical Department, University of Helsinki, in October 1956 for abdominal pains, drowsiness, nausea, vomiting and headache. For more than ten years he had suffered from ulcer symptoms which he had treated with oatmeal gruel and baking soda, of which he had taken up to 100 g daily There was no history of a previous renal disease. The patient was an asthenic, pale and somnolent man who at times was unconscious. The blood pressure was 115/80 Ophthalmological examination revealed normal conditions.

The red cell count was 5 7 mill. and the blood hemoglobin content 14 6 g/100 ml. The serum total protein content was 8.5 g/100 ml. The urine had a specific gravity of 1 014 and the reaction was acid. The sediment was normal and there was a trace of protein, but later no protein was found. The spinal fluid was normal.

The plasma bicarbonate content was 45 mEq/l the serum potassium level was 2.55 mEq/l that of sodium 125 and that of chloride 37 mEq/l The serum calcium level was 9.2 mg% The blood NPN was 138 mg% The stool guaiac test was negative.

The patient was given fluids intravenously and the electrolyte imbalance was corrected in a few days. The blood NPN was 54 mg% after three days and 32 mg% after five days. X-ray study of the upper gastrointestinal tract revealed a duodenal ulcer with hypersecretion of the stomach. The electrolyte imbalance and temporary renal insufficiency and the resulting symptoms were attributed to the excessive use of sodium bicarbonate and to vomiting. The patient was discharged with orders to avoid using sodium bicarbonate.

He was readmitted five months later because of similar symptoms of ten days duration. He admitted having continued to use baking soda as before His clinical state was very similar to that earlier The plasma bicarbonate could not be exactly measured (more than 56 mEq/l) The level of serum potassium was 2.22 and that of sodium 138 5 mEq/l The blood NPN was 67 mg% The serum calcium content was 9 6 and the phosphorus level 2.5 mg% The alkalosis with the electrolyte imbalance and azotemia were again rapidly corrected. On X ray examination the pyloric region was found to be stenotic Gastric analysis indicated a large output of hydrochloric acid. X-ray examination showed no signs of nephrocalcinosis or increased bone density The patient refused surgical treatment.

Comment

This case is an example of the complications produced by the excessive use of absorbable alkali for the relief of ulcer symptoms The condition was aggravated by vomiting which was partly due to pyloric stenosis. The case evidently corresponds to the cases described by Hardt and Rivers and others and represents the first stage of the milk-alkali syndrome.

Case 2 A 39-year-old businessman was hospitalized at the Fourth Surgical Department, University of Helsinki, in June, 1961 because of an increase in ulcer symptoms, weight loss, polyuria, malaise, and vomiting Since 1942 he had had ulcer pains, and since 1945 he had attempted symptomatic control of a duodenal ulcer by the daily ingestion of 1 1/2 l of milk and small amounts of antacids, mainly sodium bicarbonate. Since the last mentioned year he had had roentgenologic signs of pyloric stenosis and had been vomiting almost every day The history revealed no evidence of a previous renal disease. The

intake of milk was stopped but she continued to have neutralizing pills for abdominal pain.

The patient showed rapid clinical recovery from the acute phase of the disease. After a few days the serum NPN was 46 mg% around which it remained. The serum calcium level was still 13.0 mg%. The serum alkaline phosphatase was normal. The further course of the laboratory results is shown in table I. In the second hospital week the calcium-containing neutralizing agent was replaced by another antacid consisting of Al- and Mg-hydroxide (which she received until April 5). The serum calcium concentration then dropped to 9.6 mg% in a few days and generally remained normal thereafter. The urinary calcium excretion was normal on several occasions, even while the patient was receiving about 2,800 mg of calcium daily in the form of the antacid. The corrected creatinine clearance was 22 and 24 ml/min. on two occasions, and the PSP-excretion was 15 of the normal. The tubular reabsorption of phosphorus was 54 % on April 11. The phosphate clearance varied between 6 and 12 ml/min.

X-ray examination of the kidneys showed numerous small calcium shadows on both sides. A beginning band keratopathy was found in both corneas. Other soft tissue calcifications were not found. An increase in the cortical thickness of bone was indicated by a high total score of 218 (2). A bone biopsy of the iliac crest showed that the bone tissue was well mineralized. No pathological proliferation of osteoblasts or osteoclasts was noted. Some signs of eburneation were seen in the cortical part (C. A. Hernberg).

X-ray examination revealed deformity of the duodenal bulb.

A provocation test with the same calcium-containing antacid was made in the seventh week. Twenty pills daily were given during four days. On the fourth day (April 28) the serum calcium level had risen to 14.3 mg% but the urinary calcium excretion showed no increase.

The patient was discharged asymptomatic. The laboratory results remained essentially unchanged in several ambulatory examinations. A re-examination in the hospital was made after three months. The provocative test with the antacid was repeated but this time no definite increase in serum calcium was noted (July 31). The corrected creatinine clearance was 36 ml/min. and the phosphate clearance was 7 and 11 ml on two occasions. In a hospital examination in March, 1962, the degree of renal insufficiency was found to be unchanged. The serum calcium, phosphorus and alkaline phosphatase levels were normal. The degree of nephrocalcinosis was unchanged in X-rays.

Comment

The life-long excessive consumption of milk with the regular intake of sodium bicarbonate and analgesics were possibly the cause of the renal damage in this patient. After two years additional intensive use of a calcium-containing antacid a hypercalcemia with nephrocalcinosis and other signs of metastatic calcification were found to have developed. On adequate therapy the hypercalcemia disappeared but a moderate renal insufficiency remained permanent. This case represents a definite though rather mild case of Burnett's syndrome, the usually irreversible stage of the milk-alkali disorder. In this patient the progression of the disease still could be stopped. This case is of interest because of the findings suggesting osteosclerosis, a helpful criteria in the differentiation from primary hyperparathyroidism and because it seems to be the third time the syndrome is recorded in a woman.

Review of the literature

The division of the severest forms of the milk-alkali disorder into Cope and Burnett syndromes may appear unnecessary and arbitrary. However their separation seems justified and important, firstly since there are differences in the clinical pictures and secondly because Cope syndrome, in contrast to Burnett syndrome, is rapidly reversible under proper therapy and is therefore more easily separable from primary hyperparathyroidism with secondary renal insufficiency. Finally

physical examination showed a dehydrated male of asthenic body build, with a diffuse tanning of the skin. The blood pressure was 125/90 There were no macroscopic signs of corneal or conjunctival calcification. A moderately severe azotemia (creatinine 4.9 mg%) was associated with a mild hypercalcemia (11 6 to 12 7 mg %) hypokalemia (minimum 3 0 mEq/l) and a tendency towards alkalosis (plasma bicarbonate 32 and serum chloride 89 mEq/l) The levels of serum phosphorus and alkaline phosphatase were normal. The urinalysis revealed a questionable pyuria with no protein, and a specific gravity of 1 011 The renal excretions of calcium and phosphate were normal. The X rays of the skull, abdomen and chest were normal. An X-ray scoring procedure of the cortical thickness of the femur lumbar spine and hands according to the technique of Barnett and Nordin (2) gave a high normal value of 211

Upon discovery of the above biochemical disturbances a low-calcium diet was instituted together with therapy aimed at correction of the hypokalemia and preparation for surgery The course of the laboratory results is shown in table I. Gastric resection was performed on the 24th hosp tal day A stenosing duodenal ulcer was found The serum calcium level returned to normal in about a week after the operation, and the azotemia disappeared rapidly almost completely One month later the patient had gained 3 kg in weight and was feeling well.

Comment

This patient had a prolonged history of ulcer symptoms with a tendency to gastric retention and vomiting The latter without an excessive use of antacids, probably had induced a long standing mild alkalosis and hypokalemia, which together with a moderately increased consumption of milk had led to the development of Cope s syndrome the second phase of the milk-alkali syndrome After surgical correction of the basic disease the symptoms promptly disappeared but mild azotemia was still present one month later

Case 3. A 38-year-old single woman was admitted to the Third Medical Department, University of Helsinki, on March 8, 1961, because of acute illness of one day s duration with the symptoms of vomiting, mental confusion, and fever Since childhood she had been fond of milk, and since 1953 she had been taking about 3 l daily because of abdominal pains. Later she sometimes had 5 l in a day Since 1953 she also had taken about 2 tea-spoonful of sodium bicarbonate daily For about ten years she had used different drugs for headache almost every day In 1959 she was examined in a hospital. Decreased kidney function with a blood NPN of 59 mg% and secondary anemia was detected. The serum calcium level was 10 7 mg%. Urography disclosed a decreased dye excretion of both kidneys. No renal calcifications were detected at that time. A duodenal ulcer was diagnosed by X-ray but at a laparotomy no ulcer was found and a cholecystectomy was performed. The abdominal pains did not disappear after the operation. The patient began to use neutralizing pills (calcium carbonate 350 mg, ammoacetic acid 150 mg), of which she took about 20 per day

She was a slender woman with a dry and diffusely tanned skin. The lower lip showed some muscle twitchings. There was some upper abdominal tenderness on palpation. Numerous rhonchi were heard on both sides of the chest. The blood pressure was 135/75. The eyegrounds were normal.

The urine was acid with a specific gravity of 1 008, and it gave negative tests for protein and sugar The sediment was normal at the first examination, but later occasionally contained a few pus cells. The blood hemoglobin was 6.8 g/100 ml. The erythrocyte count was 1.9 mill. and that of leukocytes 15 000 with 9 5 % of non-segmented neutrophils. The tests for blood in the stools were negative. The blood NPN was 105 mg% The serum calcium level was 15 8 and that of phosphorus 2.8 mg% The plasma bicarbonate level was 15 mEq/l and that of serum potassium 3.5 mEq/l The serum sodium and chloride concentrations were normal (table 1) The chest X-ray was interpreted as normal. Respiratory infection and uremia were at first diagnosed and intravenous fluid therapy together with antibiotics was started. The

The figures refer to the number of cases with positive findings. In parentheses the number of cases in which

Azotemia	Azotemia, renal damage	Pyuria	Proteinuria	Urine pH > 6.0	Hyposthenuria	Normal urine Ca excr	Ocular les.	Calcinosis	Nephrocalcinosis (X-ray)	less bone density (X-ray)	Course improved
1	n.r.	n.r	1	n.r	1	1	0	1?	0	0	1
2	1	0	2	n.r.	2	n.r	1(1)	n.r.	0(1)	0	2
2	1?	1	2	2	2	1(1)	0	0	0(1)	0	2
1	n.r	0	0	n.r.	1	1	1	n.r.	n.r	n.r.	1
8	1	1	5	2	8	2(2)	1(7)	n.r	2(7)	n.r.	8
1	0	0	1	n.r	1	n.r.	n.r	n.r	n.r	n.r	1
1	0	0	1	n.r	n.r.	1	n.r	n.r	0	n.r.	1
1	0	1	1	n.r	1	1	1	n.r	0	0	1
1	0	n.r	1	n.r.	1	1	0	n.r	n.r.	n.r	1
1	0?	1	1	n.r	1	1	0	1?	1	n.r	1
1	0	n.r	1	n.r	n.r	1	0	n.r.	0	0	1
1	1	0	1	1	1	1	n.r	n.r	n.r	n.r.	1
1	n.r	1	1	1	n.r.	1	0	0	0	0	1
1	0	0	0	1	1	1	0	0	0	0	1
23 (23)	4(20)	5(20)	18(23)	7(15)	20(20)	12(13)	4(18)	2?(8)	3(16)	0(9)	23

The salient clinical and biochemical features of these two groups, added by our cases 2 and 3, are summarized in tables II to VI.

Most of the recorded cases were males, only one of the Cope group and three of the Barnett group being females. The mean ages of the patients and the duration of the ulcer symptoms were very similar in the two groups. In the majority of cases the ulcer was located in the duodenum. Generally there was a very long history of an excessive intake of milk and absorbable alkali (mostly sodium bicarbonate), the daily average milk consumption in the Barnett group being slightly greater (table IV).

The most common complaints (table V) largely similar in frequency in both groups (except pruritus) are attributable to hypercalcemia, alkalosis, and renal dysfunction.

Physical examination often revealed, especially in the more grave cases, a chronically ill patient with pallor, dry and often tanned skin, and dehydration. Blood pressure was usually normal or slightly elevated.

Mild to very severe azotemia existed in every instance.

Hypercalcemia was present in all cases of the Cope group and in 26 out of 33 in the Barnett group. The average calcium level was about the same in both groups (table VI), but most of the cases with a very high level were found among the clinically less severe cases (8, 13 case 3, 43 case 2). With the exception of one case in both groups, the serum

Table II Summary of the main clinical data of cases with the reversible milk-alkali (Cope's) syndrome. the information is available

Authors	No. of cases	High milk intake	Absorb. alkali intake	Hypercalcemia	Elevated serum phos.	Normal serum phos.	Normal alkal. phos.	Alkalosis
1 Kirsner (24)	1	1	1	1	1	0	↑	1
2. Dufault & Tobias (11) cases 1 and 2	2	2	2	2	0	2	1+↓	1
3. Kessler (23) cases 1 and 2	2	2	2	2	2	0	2	0
4. Ogle & Harvey (35)	1	1	1	1	1	0	↑	1
5. Scholz & Keating (41)	8	7	8	8	2	6	5+↑↑(7)	2(6)
6. Epstein (13) case 3	1	1	1	1	1	0	n.r	n.r.
7 Delcourt & Fischer (10)	1	n.r	1	1	n.r	n.r	n.r	•
8. Crabbé (8)	1	1	1	1	1	0	1	0
9 Crenshaw & Campbell (9)	1	1	1	1	0	1	n.r	0
10. Frank & Greenspan (15)	1	1	1	1	0	1	↑	0
11 Grégoire et al. (19)	1	1	1	1	0	↓	n.r	1
12. Slot (42)	1	1	n.r	1	0	1	n.r.	•
13. Van Ypersele de Strihou & Crosnier (45) case 2	1	1	1	1	0	1	1	1
14. Punsar & Somer case 2	1	1	1	1	0	1	1	1
Total	23	21(22)	22(22)	23(23)	8(22)	13(22)	11(17)	8(20)

n.r – Not recorded ↑ – Elevated ↓ – Decreased – Conjunctivitis

Cope was the first to observe hypercalcemia, the leading symptom in both syndromes.

Therefore (and also in the hope that additional characterizing signs for the proper Burnett's syndrome would be found) the authors classified the 54 cases with the milk alkali syndrome published since the report of Burnett et al. in 1949 into these two categories. As differentiating criteria were used the two leading characteristics of Burnett's syndrome, namely the existence of band keratopathy or other metastatic calcification, combined with an irreversibility of the clinical condition.

Of the 54 cases fifteen patients with hypercalcemia did not have signs of metastatic calcification. Each of them also showed rapid clinical recovery Seven further cases with either minimal corneal calcification or urolithiasis also showed rapid improvement on appropriate therapy These 22 cases were considered to represent examples of Cope's syndrome. (To this group could also be added the large series of 35 cases of Wenger et al. (48) observed among 3,300 hospitalized ulcer patients. This well analyzed group, which represents episodes of acute intoxication caused by an intensive application of the Sippy program, is not considered here.)

In the category of Burnett's syndrome thus remained 32 cases, who all showed definite metastatic calcifications and either an irreversible renal failure or a slow amelioration of the clinical condition.

drome. The figures refer to the number of cases with positive findings. In parentheses the number of cases

Assum. renal damage	Pyuria	Prot.-uria	Urine pH > 6.0	Hyposthenuria	Normal urine Ca excr	Ocular les.	Calcinosis	Nephrocalcinosis (X-ray)	Incr bone density (X-ray)	Course: Improved	Course: Died	Autopsy
1	4	4	2(3)	5	5(5)	6	5	1	2	1	3	2
n.r	0	1	1	1	n.r	1	1	0	n.r	1	—	—
0	0	1	1	1	n.r	1	1	0	0	1	—	—
0	1	1	n.r	1	1	1	1	*0	0	—	1	1
2	2	2	n.r	2	1+†	2	2	2	0	1	1	1
1	1	1	n.r	1	1	1	1	1	0	1	—	—
0	0	1	1	1	1	1	1	n.r	n.r	1	—	—
1	0	1	1	1	1	1	n.r.	*0	1	—	1	1
0	1	1	1	1	1	1	1	1	0	1	—	—
n.r	1	2	2	1	2	2	2	2	0	n.r	1	—
1?	0	1	n.r.	1	1	0	1	*0	0	—	1	1
n.r	1	1	1	1	1	0	1	0	0	1		—
n.r	n.r	n.r	1	1	†	n.r.	0	1	0	1		—
n.r	1	1	1	1	1	1	n.r.	0	n.r	1	—	—
n.r	1(1)	1(1)	n.r	2	1+†	2	2	2	0(1)	1	1	1
1	0	1	n.r	1	1	1	1	0	0	—	1	1
n.r	1	1	n.r	n.r	1	1	n.r	1	1	1	—	—
1	1	0	1	1	1	0	0	1	0	1?	—	—
0	1	1	0	1	1	1	1	1	1	1	—	—
1(3)	3	2(3)	4	4	2+†(5)	2	3	1(2)	0	4	—	—
0	1	1	n.r	n.r.	1	1	0	0	1	1	—	—
0	1	1	1	1	1	1	0	1	1	1	—	—
9(23)	21(31)	?8(30)	18(20)	29(31)	23(29)	27(32)	24(30)	15(30)	7(29)	20(32)	12(32)	8

* Nephrocalcinosis demonstrated histologically

† Improved but died in hematemesis.

group, and in two-thirds of the cases with Burnett syndrome. However bacteriuria was not common. Some cases in the Burnett group showed slight hematuria. Proteinuria of variable degree was found in the vast majority of the cases in both categories. Urine pH, where recorded, was abov 6.0 in half of the cases with Cope syndrome and even more alkaline in the Burnett group. Hyposthenuria was almost rule in both groups, and the reduced tubular function was often also reflected in the impaired PSP clearance.

According to the definition, the urinary calcium excretion was normal except in a few

Table III Summary of the main clinical data of cases with the irreversible milk-alkali (Burnett's) syn- in which the information is available

Authors	No. of cases	High milk intake	Absorb. alkali intake	Hypercalcemia	Elevated serum phos.	Normal serum phos.	Normal alkal. phos.	Alkalosis	\ ormal
1 Burnett et al. (3)	6	6	6	5	4	2	4+↑(5)	5	6
2. McQueen (29)	1	1	1	1	0	1	1	1	1
3 Miller et al. (30)	1	1	1	1	0	1	1	1	1
4 Wermer et al. (49)	1	1	1	1	0	1	1	0	1
5 Dufault & Tobias (11) cases 3 and 4	2	2	2	2	2	0	2	1	2
6. Dworetzky (1)	1	1	1	0	0	1	1	0	1
7 Folz (14)	1	1	1	1	0	1	1	0	1
8. Kyle (26) case 1	1	1	1	1	1	0	1	0	1
9. Rodnan & Johnson (39)	1	0	1	0	1	0	1	1	1
10. Snapper et l. (43)	2	1	2	1	2	0	↑(1)	0(1)	2
11 Holten & Lundbæk (21)	1	1	1	0	1	0	1	0	1
12 Kessler (23) case 3	1	1	1	1	0	1	1	0	1
13. Schneider (40) case 1	1	1	1	1	0	1	n.r	n.r	1
14 Wimmer (50)	1	1	1	1	1	0	1	0	1
15. Poppel & Zeitel (36)	2	2	2	2	1	1	1(1)	0	2
16 Case no. 42441 of the Mass. Gen. Hosp. (5)	1	1	1	1	1	0	1	n.r.	1
17 Milliez et al. (31)	1	n.r	1	1	1	0	n.r	1	1
18. Lindenschmidt & Piening (8)	1	1	1	0	0	↓	1	0	1
19. Rifkind et al. (38)	1	0	1	1	1	0	1	0	1
20. Randall et al. (37)	4	4	4	4	0	4	2+↑↑	0	4
21 Van Ypersele de Strih u & Crosnier (45) case 1	1	1	1	0	1	0	↑	1	1
22 Punsar & Somer case 3	1	1	1	1	0	1	1	0	1
Total	33	29(32)	33(33)	26(33)	17(33)	15(33)	23(28)	11(30)	33(33)

Further course and aut psy findings of case 3 of Burnett et al. are reported in New Engl. J. Med. *255* 870, 1956.

phosphorus level was normal or elevated the proportion of cases w th increased levels being greater in the Burnett group Characteristically alkaline phosphatase was normal or low in both categories, except in some cases which, because of other evidence were regarded as examples of the milk-alkali syndrome Generally there was a slight tendency towards alkalosis.

A previous renal disease or at least a suspicion was present in about one-third of the cases in the Burnett category and less frequently in the Cope group.

Pyuria was found in a few cases of the Cope

drome. The figures refer to the number of cases with positive findings. In parentheses the number of cases

Anaemia, renal damage	Pyuria	Proteinuria	Urine pH > 6.0	Hyposthenuria	Normal urine Ca excr	Ocular les	Calcinosis	Nephrocalcinosis (X-ray)	Incr bone density (X-ray)	Course		Autopsy
										Improved	Died	
1	4	4	2(3)	5	5(5)	6	5	1	2	1	5	2
n.r.	0	1	1	1	n.r	1	1	0	n.r	1	—	—
0	0	1	1	1	n.r	1	1	0	0	1	—	—
0	1	1	n.r	1	1	1	1	0	0	—	1	1
2	2	2	n.r	2	1+†	2	2	2	0	1	1	1
1	1	1	n.r	1	1	1	1	1	0	1	—	—
0	0	1	1	1	1	1	1	n.r	n.r	1	—	—
1	0	1	1	1	1	1	n.r.	0	1	—	1	1
0	1	1	1	1	1	1	1	1	0	1	—	—
n.r.	1	2	2	1	2	2	2	2	0	n.r	1	—
1?	0	1	n.r.	1	1	0	1	0	0	—	1	1
n.r	1	1	1	1	1	0	1	0	0	1	—	—
n.r.	n.r.	n.r.	1	1	†	n.r	0	1	0	1	—	—
n.r.	1	1	1	1	1	1	n.r.	0	n.r	1	—	—
n.r.	1(1)	1(1)	n.r	2	1+†	2	2	2	0(1)	1	1	1
1	0	1	n.r	1	1	1	1	0	0	—	1	1
n.r.	1	1	n.r.		1	1	n.r	1	1	1	—	—
1	1	0	1	1	1	0	0	1	0	1?	—	—
0	1	1	0	1	1	1	1	1	1	1	—	—
1(3)	3	2(3)	4	4	2+†(3)	2	3	1(2)	0	4	—	—
0	1	1	n.r	n.r	1	1	0	0	1	1	—	—
0	1	1	1	1	1	1	0	1	1	1	—	—
9(23)	21(31)	26(30)	18(20)	29(31)	25(29)	27(32)	24(30)	15(30)	7(29)	20(32)	12(32)	8

Nephrocalcinosis demonstrated histologically

Improved but died in hematemesis

group, and in two-thirds of the cases with Burnett syndrome. However bacteriuria was not common. Some cases in the Burnett group showed slight hematuria. Proteinuria of variable degree was found in the vast majority of the cases in both categories. Urine pH, when recorded, was above 6.0 in half of the cases with Cope syndrome and even more alkaline in the Burnett group. Hyposthenuria was almost a rule in both groups, and the reduced tubular function was often also reflected in the impaired PSP clearance.

According to the definition, the urinary calcium excretion was normal except in few

Table III Summary of the main clinical data of cases with the irreversible milk-alkali (Burnett's) syndrome in which the information is available

Authors	No. of cases	High milk intake	Absorb. alkali intake	Hypercalcemia	Elevated serum phos.	Normal serum phos.	Normal alkal. phos.	Alkalosis	Azotemia
1 Burnett et al. (3)	6	6	6	5	4	2	4+↑(5)	5	6
2. McQueen (29)	1	1	1	1	0	1	1	1	1
3 Miller et al. (30)	1	1	1	1	0	1	1	1	1
4 Wermer et al. (49)	1	1	1	1	0	1	1	0	1
5. Dufault & Tobias (11) cases 3 and 4	2	2	2	2	2	0	2	1	*
6. Dworetzky (12)	1	1	1	0	0	1	1	0	1
7 Folz (14)	1	1	1	1	0	1	1	0	1
8. Kyle (26) case 1	1	1	1	1	1	0	1	0	1
9. Rodnan & Johnson (39)	1	0	1	0	1	0	1	1	1
10. Snapper et al. (43)	2	1	2	1	2	0	↑(1)	0(1)	2
11 Holten & Lundbaek (21)	1	1	1	0	1	0	1	0	1
12. Kessler (23) case 3	1	1	1	1	0	1	1	0	1
13. Schneider (40) case 1	1	1	1	1	0	1	n.r	n.r	1
14 Wissmer (50)	1	1	1	1	1	0	1	0	1
15 Poppel & Zeit 1 (36)	2	2	2	2	1	1	1(1)	0	2
16. Case no. 42441 of the Mass. Gen. Hosp. (5)	1	1	1	1	1	0	1	n.r	1
17 Milliez et al. (31)	1	n.r	1	1	1	0	n.r	1	1
18. Lindenschmidt & Piening (28)	1	1	1	0	0	↓	1	0	1
19. Rifkind et al. (38)	1	0	1	1	1	0	1	0	1
20. Randall et al. (37)	4	4	4	4	0	4	+↑↑	0	4
21 Van Ypersele de Strihou & Crosnier (45) case 1	1	1	1	0	1	0	↑	1	1
22. Punsar & Somer case 3	1	1	1	1	0	1	1	0	1
Total	33	29(32)	33(33)	26(33)	17(33)	15(33)	23(28)	11(30)	33(33)

Further course and autopsy findings of case 3 of Burnett et al. are reported in New Engl. J Med. *255* 870 1956.

phosphorus level was normal or elevated the proportion of cases with increased levels being greater in the Burnett group Characteristically alkaline phosphatase was normal or low in both categories, except in some cases which, because of other evidence, were regarded as *examples of* the milk-alkali syndrome Generally there was a slight tendency towards alkalosis.

A previous renal disease or at least a suspicion was present in about one-third of the cases in the Burnett category and less frequently in the Cope group.

Pyuria was found in a few cases of the Cope

In all eight cases in which autopsy was performed, severe nephrocalcinosis and soft tissue calcification on other sites were found (e.g., calcification of subcutis, periarticular tissue, tendons, lungs, arteries, dura) In two cases no parathyroid tissue was found. In six cases the parathyroid glands showed a mild hyperplasia, which generally was attributed to the presence of a chronic kidney disease (e.g. 49) and was not regarded as the cause of the disorder

Summary of the review

Several facts emerge from the analysis of the literature presented above. Cases with the milk-alkali syndrome usually have a long history of ulcer symptoms and intake of absorbable alkali, but the length of history or the type of treatment do not seem to be the determining factors in the severity of the disease. In general, patients with Burnett's syndrome had had greater daily milk consumption, and a renal disease was slightly more common in the case histories in this group

The division of cases with the milk-alkali syndrome into those with the transient and those with the chronic form of the disease could usually be made already on the basis of absence or presence of metastatic calcifications, this generally manifesting itself either in the form of band keratopathy or roentgenologically discernible soft tissue calcification, or both. Apparently the occurrence of metastatic calcification indicates that the disease has progressed to an irreparable stage. The transient Cope's syndrome carries in itself a good prognosis, whereas one third of the cases with significant calcification had a fatal outcome and in the remaining cases a permanent azotemia often remained.

Hypercalcemia was a constant finding in Cope's syndrome but was absent in about 20 per cent in the Burnett group. The serum phosphorus level was generally higher in the latter group. These observations might be explained by the difference in the severity of the renal lesion, which also was indicated by the common occurrence of nephrocalcinosis and pyuria in Burnett's syndrome. In spite of renal insufficiency an alkalotic tendency was often found in both groups, but it was not obligatory and was absent in cases with more advanced renal insufficiency

X ray signs of an increased mineralization of the bones were found in about one-fourth of the cases with Burnett's syndrome, this finding being not recorded in the cases of the Cope group.

Discussion

The name milk-alkali syndrome is often used in connection with the complications resulting from excessive antacid treatment of peptic ulcer This name is practical in indicating the circumstances in which the condition commonly develops, and therefore it seems to be suitable as a general term. Its utilization is not invalidated by the occurrence of a few cases (e.g., 38, 39 41 case 3 43 case 2) without a history of abundant (more than 1 l) daily milk intake. However for the reasons discussed above, a more exact definition is necessary to indicate better the stage of development of the disease. For this purpose the names "reversible" milk-alkali (i.e., Cope's) syndrome and respectively "irreversible" milk alkali (i.e., Burnett's) syndrome, might be more appropriate, as proposed by some authors (38, 48)

The pathogenesis of the milk-alkali syndrome is somewhat obscure. Already the development of hypercalcemia imposes complicated pathogenetic problems.

Table IV Comparison between Cope's and Burnett s syndromes

	Cope s syndrome (23 cases)	Burnett's syndrome (33 cases)
Mean age (yrs)	48 (23)	52 (32)
Duration of ulcer symptoms (yrs)	16 (22)	18 (31)
Duration of excessive milk and alkali intake (yrs)	15 (13)	13 (28)
Average daily milk intake (l)	1.9 (15)	2.7 (20)
Blood pressure (mm Hg)	148/97 (20)	144/89 (20)

Table V Comparison between Cope s and Burnett s syndromes Frequency of main subjective symptoms

	Cope's syndrome (23 cases)		Burnett's syndrome (33 cases)	
Anorexia	22%	(5)	18%	(6)
Asthenia	22%	(5)	30%	(10)
Mental symptoms	30%	(7)	18%	(6)
Muscle ches	13%	(3)	33%	(11)
Nausea and vomiting	74%	(17)	45%	(15)
Polydipsia	13%	(3)	27%	(9)
Polyuria (nocturia)	26%	(6)	55%	(18)
Pruritus	—	(1)	40%	(13)

Table VI Comparison between Cope s and Burnett s syndromes Laboratory data

	Cope s syndrome (23 cases)		Burnett syndrome (33 cases)	
Serum Ca (mg/100 ml)	13.7	(23)	13.1	(33)
Serum P (mg/100 ml)	4.2	(21)	5.4	(31)
Azotemia BUN (mg/100 ml)	103	(17)	88	(16)
Azotemia NPN (mg/100 ml)	87	(5)	112	(14)
Plasma bicarbonate (mEq/l)	30.5	(20)	30.8	(29)
Serum Na (mEq/l)	136	(8)	137	(12)
Serum chloride (mEq/l)	84	(10)	91	(17)
Serum potassium	—		4.1	(11)

cases where, despite a slight temporary hypercalciuria, the diagnosis of the milk alkali syndrome was made because of other evidence.

In the cases of the Cope group, ocular calcifications were typically absent except in four cases with minimal ocular calcium deposits. From the series were excluded cases with soft tissue calcifications characteristic of Burnett s syndrome.

In cases considered to represent the true Burnett s syndrome, ocular lesions in form of band keratopathy were present in 27 of 32 cases. Twenty out of the 30 cases with Burnett s syndrome in which the information was given, exhibited X-ray or histological evidence of nephrocalcinosis. Other soft tissue calcifications were present in 24/30 cases.

X-ray examination of the bones was considered normal in all cases of the Cope group where reported. On the contrary in 7 out of 29 cases of the Burnett category some evidence of an increased bone mineralization was found by X-ray In three of these, including our own case No. 3, the roentgenological diagnosis of osteosclerosis was confirmed by bone biopsy (31 38)

All cases with milder clinical symptoms showed a rapid improvement on proper therapy Hypercalcemia disappeared quickly but signs of renal insufficiency persisted for several weeks in some of the cases. In contrast a third of the patients with Burnett's syndrome died within a few years, usually of progressive renal failure. In the remaining two-thirds of cases clinical recovery occurred, but in many of them complete correction of renal insufficiency was not achieved. Despite subjective improvement there occurred in some cases a further increase in the already elevated serum calcium concentration, together with a pronounced hypercalciuria (37). Simultaneously a deterioration in renal function tests could also be seen. In some cases soft tissue calcification showed roentgenologically a diminution, but the band keratopathy usually remained

with calcification in other sites and, simultaneously with an irreparable form of the disease, i.e., this sign generally indicated the diagnosis of Burnett's syndrome. In certain cases, the ocular deposition of calcium occurred and/or disappeared rapidly (e.g. 37 case 3, 48). In four instances only despite (minimal) band keratopathy the prompt clinical response to adequate treatment necessitated placing the case into Cope's category.

In the differential diagnosis of the milk-alkali syndrome are to be considered all states associated with renal insufficiency alkalosis, hypercalcemia, or soft tissue calcification. A consideration of the entire clinical picture will usually serve to differentiate these other conditions. The main difficulty lies in the distinction between Burnett's syndrome and primary hyperparathyroidism in a patient without bone disease and with an active peptic ulcer and renal insufficiency. There are reports of several cases of primary hyperparathyroidism with renal failure and ulcer confused with the milk-alkali syndrome (1 4 26 case 2, 33 40 case 2, 46). The correct diagnosis is desirable but without a surgical exploration for parathyroid abnormality not possible after the renal insufficiency is far advanced.

The presence of peptic ulcer with a history of milk and alkali treatment is not always connected with the etiology of hypercalcemia as a high prevalence of peptic ulcer has been noted in patients with primary hyperparathyroidism. The typical findings in hyperparathyroidism are hypercalciuria and hypophosphatemia, whereas in the milk-alkali syndrome normal calcium excretion and normal or elevated serum phosphorus levels are usually found. Several other features may be of help in the diagnosis of the milk alkali syndrome, such as a normal serum alkaline phosphatase level, the presence of mild alkalosis, and a tendency towards an alkaline urine reaction. Special laboratory tests have been used in the diagnosis of hyperparathyroidism, e.g., the intravenous calcium loading test and the measurement of the renal tubular reabsorption of phosphate (27 44) but their value in the differential diagnosis of the milk alkali syndrome is still debatable. The return of the serum calcium level to normal and the general clinical improvement by conservative means speak against hyperparathyroidism. The above mentioned diagnostic criteria usually become valueless with progressing severe renal failure. Also the absence of signs of increased bone resorption does not exclude hyperparathyroidism, whereas histologically or roentgenologically demonstrable osteosclerosis excludes the possibility of hyperparathyroidism. The considerable frequency of increased bone mineralization in Burnett's syndrome has not been previously mentioned in the literature. It seems that more attention should be paid to this sign.

The treatment of the milk alkali syndrome has consisted principally of a low calcium diet and discontinuance of absorbable alkali. Usually high fluid therapy non-absorbable alkali (mostly in the form of aluminium hydroxide gel and magnesium trisilicate) and antispasmodics have been prescribed. With this therapeutic regime, the clinical state of patients with a favourable outcome may improve rapidly but in advanced cases signs of renal insufficiency have remained permanent.

The fundamental significance of this, often iatrogenic, disorder lies in its rela

The effect of the heavy ingestion of a milk diet containing large amounts of calcium and phosphorus on the serum calcium level is unsettled Only a fraction of ulcer patients develop this syndrome. The antacids used by the milk-alkali patients have often included calcium but in the majority sodium bicarbonate was the chief alkalizing agent In any event the amount of calcium ingested seems to have been higher than normal in cases with the milk-alkali syndrome, with the exception of three cases (8 10 43 case 2) The possibility of an increased calcium absorption in these patients remains open. It is known that gastric acidity may play a role in the resorption of calcium (34) but there is no convincing evidence that hyperacidity facilitates calcium absorption nor is there any absolute proof of the probable existence of excessive gastric acidity in patients developing the milk alkali syndrome. It can be speculated that factors such as increased sensitivity to vitamin D might play a role in the development of hypercalcemia in this syndrome.

An alkalotic period as a result of excessive alkali ingestion and/or chronic vomiting probably belongs to the pathogenesis of this disorder as an essential feature (37 48) The direct effect of alkalosis on the renal excretion of calcium is still open Alkalosis may impair renal function, possibly by producing potassium depletion whereby the renal excretion of calcium may become diminished (26) Alkalosis probably promotes the intratubular precipitation of calcium salts thereby enhancing renal damage and further impairing the renal excretion of calcium An excessive load of calcium upon the kidney is known to be capable *per se* of producing a reduction in renal function (16)

It has been suggested that pre-existing renal disease is an important predisposing factor in the development of the milk-alkali syndrome (e.g 48) A surprisingly high proportion of the reported cases had a history suggesting antecedent kidney disease. It seems that the presence of previous renal disease is not a prerequisite, but may be a promoting factor in the genesis of this syndrome.

Nephrocalcinosis has always been observed when histological examinations of renal tissue have been made in cases with Burnett s syndrome (although not necessarily evident by X ray) In addition to hypercalcemia and alkalosis, the intratubular deposition of calcium might also be facilitated by dehydration caused by vomiting and polyuria and the resulting low rate of urine. The complete mechanism of the development of nephrocalcinosis however remains unsolved, as also is the case with metastatic calcification in other sites, so commonly found in Burnett s syndrome.

A clinically readily detectable physical sign for the determination of the severity of the disease is to be found in the eyes. The ocular lesions, occurring in hypercalcemia from any cause (47) are of two types. In the first there are hazy calcium deposits in the cornea running paralimbally This lesion designated as band keratopathy may easily be mistaken for arcus senilis, but it is usually broader and more centrally located than the latter The other type of ocular lesion consists of small glass-like conjunctival particles. A slit lamp examination is usually required for the more exact identification of both lesions although they can often be recognized without it. In reviewing the literature it soon became clear that the finding of ocular calcification was usually connected

with calcification in other sites and, simultaneously with an irreparable form of the disease, i.e. this sign generally indicated the diagnosis of Burnett's syndrome. In certain cases, the ocular deposition of calcium occurred and/or disappeared rapidly (e.g. 37 case 3 48). In four instances only despite (minimal) band keratopathy the prompt clinical response to adequate treatment necessitated placing the case into Cope's category.

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The fundamental significance of this, often iatrogenic, disorder lies in its rela-

tion to the therapy of peptic ulcer With conservative treatment it is important to be on the alert for the potentially serious hazards of prolonged excessive intake of milk and absorbable alkali, and to initiate a search for hypercalcemia, alkalosis and renal insufficiency in patients presenting such a history To prevent the occurrence of complications of this kind the choice of a proper antacid is essential In this connection it might be well to recall the characteristics of a good antacid the neutralizing effect should be strong and prolonged, with no ensuing acid rebound and no general alkalosis in addition to the other desirable good qualities of causing no obstipation laxation or local irritation and of being tasteless (32) A further requirement should possibly be that an antacid must contain no calcium salts.

Summary

The milk alkali syndrome is a disorder with diverse clinical and biochemical manifestations. It includes three known clinical syndromes, each of which is illustrated by the authors by a case report. An analysis is made of the features in all of the cases which have been reported in the literature since the first description of the gravest form of the milk-alkali syndrome (Burnett's syndrome) A distinction between the latter and the less advanced stage (Cope's syndrome) can chiefly be made by the presence or absence of signs of metastatic soft tissue calcification This calcification is often connected with a poor prognosis in the form of an irreversible renal failure. The value of a finding of increased bone mineralization in the differential diagnosis from primary hyperparathyroidism is emphasized

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Addendum

Since the submission of the manuscript two additional reports of Burnett's syndrome have come to the authors' notice (D[illegible], N. J., V[illegible], J. V. & Engel, F. L. Amer. J. Med. 33 88, 1962; Virta, J. & Sharp E. Rhode Island med. J. 45 91, 1962). It is noteworthy that the case of Virta and Sharp showed X-ray signs of increased calcium content of bones.

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Cytochemical Studies of Glycogen Content of Lymphocytes in Lymphatic Leukaemia and Reactive Lymphocytosis

By

Östen Björkstrand

The occurrence of glycogen in blood cells has lately been studied with the so-called periodic acid-Schiff (PAS) staining technique according to McManus (5) Hotchkiss (4) and Gomori (2) In healthy individuals most lymphocytes entirely lack glycogen. However in a small number of lymphocytes glycogen can be demonstrated mostly as a few minute, well-defined, dark bluish granules scattered in the cytoplasm but sometimes arranged in one or more concentric rings along the border of the cytoplasm.

Wachstein (8) was one of the first scientists who studied the occurrence of glycogen in lymphocytes in lymphatic leukaemia. In this disease he found that the glycogen activity was the same as in healthy individuals. Wislocki et al. (9) reported at about the same time that the number of lymphocytes that showed PAS-positive granules increased in chronic lymphatic leukaemia. Astaldi and Verga (1) confirmed this observation. They also found that the glycogen activity was significantly greater in immature than in mature lymphatic leukaemia. Quaglino et al. (7) could not later verify this observation.

Mitus et al. (6) showed that the increase in glycogen activity was not specific for malignant diseases in the lymphocytic blood system but could be seen in non-malignant disorders too

The purpose of the present work was to study the glycogen activity in lymphocytes in mature and immature lymphatic leukaemia and in so-called reactive lymphocytoses and to find out if from this investigation any conclusions could be formulated with regard to the differential diagnosis between these two conditions.

Method

Grönwall-Kohn's technique (3) modified in the following manner

Air-dried films of bone marrow were

1 Fixed in formalin-ethanol (1 part 35 formalin (Merk pro analys. neutral) 9 parts 95 % ethanol) for 5 min.

Where the reagents have been changed the new composition has been given in parenthesis.

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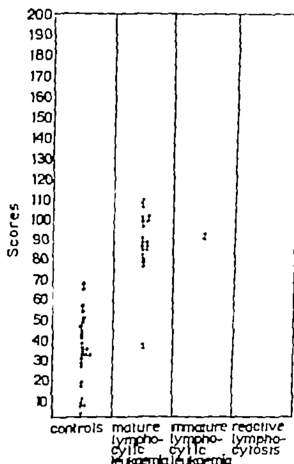


Fig 1 Glycogen activity in mature and immature lymphocytic leukaemia and in reactive lymphocytosis.

2 Rinsed in running water for 15 min.

3 Placed in periodic acid solution for 8 min.

4 Rinsed in distilled water

5 Placed in Schiff's fuchsin solution for 30 min.

6. Rinsed in distilled water

7 Rinsed 4—5 times in sulphite rinse solution.

8 Rinsed in distilled water for 5 min.

9 Counterstained in malachite green (0 01g/100 ml distilled water) for 0.5 min.

Control

Films of bone marrow exposed to salivary digestion in room temperature for 30 min. before treatment with periodic acid.

Scoring

The activity of glycogen was graded as follows

0 = cells with no PAS-positive granules.

1 = cells with one or more granules scattered or arranged in one ring along the border of the cytoplasm.

2 = cells with granules arranged in two concentric rings.

3 = cells with granules arranged in three or more concentric rings or clumps of PAS-positive material.

At the estimate some consideration has been taken to the size and the colour intensity of the granules.

Score

One hundred lymphocytic cells counted with a possible range of 0 to 300.

Material

Controls

Thirty-one patients without signs of disease of the lymphocytic blood system. Among them were patients with iron deficiency anaemia.

Leukaemia

Forty-three patients with acute or chronic lymphatic leukaemia. The diagnosis was based upon clinical findings, bone marrow examinations and mostly pathologic anatomical examinations of material from biopsy or autopsy. Eight of the 43 cases were classified as immature lymphatic leukaemia, in whom the majority of the lymphatic cells were blast-forms.

Reactive lymphocytosis

Six patients with absolute lymphocytosis (8—15,000). All of them showed a clinical picture as in infectious mononucleosis. Three had pathological Paul Bunnel- and OCH-reactions.

Results

Controls

Scores between 3 and 77. Mean score 36.

Mature lymphatic leukaemia

Scores between 12 and 176. Mean score 96

Immature lymphatic leukaemia

Scores between 9 and 98. Mean score 66

Reactive lymphocytosis

Scores between 34 and 155 Mean score 98.

In repeated stainings the reproduction with regard to the PAS-activity was good. There was no relationship between the score and the number of lymphocytes in the peripheral blood

Discussion

The study confirms the observation that lymphocytes in bone marrow in lymphatic leukaemia have a high glycogen activity Immature forms do not differ from mature forms in this respect.

Eleven out of the 43 patients had a normal score. In all these 11 cases but 3 the leukaemia was untreated. Treatment of the leukaemia is said to reduce the glycogen content of the cells (7) Nor did this group differ from the rest of the cases of leukaemia with regard to the number of lymphocytes in the peripheral blood. (2,000—250 000)

In reactive lymphocytosis the lymphocytes have as high glycogen content as in lymphocytic leukaemia.

A high score speaks in favour of a disturbance of the lymphocytic blood system but does not differentiate between malignant and non-malignant disorders. A low or normal score speaks to some extent in favour of a non-malignant disorder

Summary

The occurrence of glycogen was studied with the PAS staining technique in lymphocytes of the bone marrow from 43 patients with lymphatic leukaemia and from 6 patients with reactive lymphocytosis (infectious mononucleosis) As compared with controls the PAS-activity was high in the lymphocytes from these two groups of patients. A high score speaks in favour of a disturbance of the lymphatic blood system but does not differentiate between malignant and non-malignant disorders. A low or normal score speaks to some extent in favour of a non-malignant disorder

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Periodic-acid-Schiff Staining and Classification of So-called Undifferentiated Reticuloses

By

Östen Björkberg

Leukoses where the primitive cells in the bone marrow could not be differentiated with the May-Grünwald-Giemsa and the peroxidase staining techniques are considered to be undifferentiated reticuloses. The purpose of the present work was to study if it was possible with the help of the periodic-acid-Schiff (PAS) staining technique to separate these so-called undifferentiated reticuloses in "lymphocytic" and "not lymphocytic" forms and to ascertain whether these two groups differed in regard to clinical findings and therapy

Method and material

The PAS staining technique and the estimate of the results were described in a preceding article (1) Films of bone marrow from 13 patients with peroxidase negative, so-called undifferentiated reticuloses were examined.

Results and discussion

In table I are shown the results of bone marrow films stained with the May Grünwald-Giemsa and the PAS techniques together with clinical and pathologic-anatomical data of all patients of the series.

In 7 of the examined films the undifferentiated cells showed no form of PAS activity In the remaining 6 the glycogen content in the cells was of typical "lymphocytic" type, which means that it occurred as well defined, dark blue red granules scattered or more often arranged in one or more concentric rings in the cytoplasm. Sometimes the activity was in the form of rather big, bluish clumps in the cytoplasm or sometimes in the nucleus. The PAS activity was high in all 6 cases and quite comparable with that of lymphatic leukaemia (1) The scores varied between 80 and 232. Mean score 158. In repeated stainings the reproduction was good. Three of the cases were followed during six months. The PAS activity was unchanged and consequently had no relationship to any special phase in the course of the disease.

With the help of the PAS staining technique it was accordingly possible to divide the undifferentiated reticuloses into two groups one "not lymphocytic"

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Table I Estimate of the May-Grünwald-Giemsa staining technique and the PAS staining technique of bone marrow films clinical findings effect of treatment and pathologic-anatomical examination in 13 patients with so-called undifferentiated reticuloses

Case	May-Grünwald Giemsa staining technique	PAS staining technique (scores)	Enlargement of lymphatic glands	Enlargement of spleen	Duration of disease (mos)	Effect of treatment	Pathologic anatomical examination
1	92% undifferentiated mononuclear cells (myeloblasts?)	0	Some	++	12	Prednisolone good temporary effect	Undifferentiated reticulosis
2	91% undifferentiated mononuclear cells	0	General	++	6	Prednisolone no effect	Monocytic leukaemia
3	41% undifferentiated mononuclear cells	0	No	++	2	Prednisolone good temporary effect	Not performed
4	27% undifferentiated mononuclear cells	0	General	++	2	"Sendoxan": no effect	Reticulosis
5	29% undifferentiated mononuclear cells	0	No	–	12	Prednisolone good effect?	Primitive reticulosis
6	73% undifferentiated mononuclear cells	0	No	–	6	Prednisolone no effect	Undifferentiated reticulosis
7	91% undifferentiated mononuclear cells	0	N	++	3	Prednisolone no effect	Not performed
8	91% undifferentiated mononuclear cells	227	General	+	4	Prednisolone good temporary effect	Undifferentiated reticulosis
9	97% undifferentiated mononuclear cells	103	General	++	12	Prednisolone + mercaptopurine good temporary effect	Not performed
10	53% undifferentiated mononuclear cells (lymphocytic?)	80	Some	–	>24	Prednisolone good temporary effect	Not performed
11	Reticular cells (myelocytic?)	252	No	?	6	Prednisolone doubtful effect	Not performed
12	65% mononuclear cells (lymphocytic?)	108	General	?	12	Prednisolone: doubtful effect	Lymphosarcoma?
13	21% undifferentiated mononuclear cells (lymphocytic?)	108	General	++	24	Prednisolone: good temporary effect	Not performed

and one "lymphocytic" It is known that young myelocytic cells (myeloblasts) react on PAS staining as those in the first group (2)

In 3 of the 6 cases in the "lymphocytic" group there were in the May-Grünwald-Giemsa staining technique some cells that were suspected to be of lymphocytic type. In the other group there were no such cells.

In regard to the clinical picture and the course of the disease there was no real difference between the patients in the two groups. However enlargement of the lymphatic glands was noticed in 5 of the 6 "lymphocytic" cases and only in 3 of the 7 not lymphocytic" cases. All patients but one, who is still alive died within 2 years after the beginning of the disease.

Pathologic-anatomical examination when performed could not differentiate the young cells. However in one case in the "lymphocytic" group lymphosarcoma was suspected. In one case in the not lymphocytic" group monocytic leukaemia was suspected

The effect of treatment with prednisolone and mecaptopurine respectively was equal in the two groups. However the estimate of this effect was difficult, as blood transfusions were given t the same time. Besides, one can expect a priori that the effect of treatment with prednisolone-mecaptopurine in these relatively acute diseases is fairly independent of the myelocytic or lymphocytic character of the reticulosis.

Summary

Bone marrow films from 13 patients with so-called undifferentiated reticuloses were examined with the PAS staining technique. In regard to this technique, it was possible to divide the 13 cases into two groups one group of 6 cases where the undifferentiated cells showed a typical "lymphocytic" PAS activity and another group of 7 cases where the cells were entirely PAS negative. The May Grünwald-Giemsa staining technique, clinical and pathologic-anatomical examinations confirmed that this division into "lymphocytic" and not lymphocytic" forms was adequate. The future will show if this subdivision of the so-called undifferentiated reticuloses has any practical clinical value.

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Selection in Diabetes in Modern Society

By

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The epidemiology of diabetes mellitus presents several urgent problems. That the incidence of diabetes has been rapidly increasing in western civilizations since the change of the century is beyond doubt, but it is not clear whether this may be explained fully as a result of the changing age composition of the population, with increased representation of older age classes in combination with the lengthening of the life span of diabetics after the introduction of effective therapeutics, in particular of insulin. On the other hand it cannot be ruled out that the increase of frequency reflects a real elevation of the morbid risk be it due to environmental or to genetic factors. Thus it might be feared that the growing number of juvenile diabetics kept alive through their reproductive period of life would mean an alleviation of the selective pressure against diabetes and imply an increase of the frequency of diabetic genes in the gene pool of the population.

Aschner and Post (1) have discussed this problem on the basis of data procured from earlier family studies, published by different authors. From the fact that the number of offspring in families having at the least one diabetic child is consistently larger if one of the parents also is diabetic and even more so if both parents are diabetics, it is concluded that the carriers of the diabetes gene or genes have a selective advantage, and it is calculated that a doubling of genetically determined diabetes may be expected in the course of five to ten generations.

For different reasons, however, it is more than doubtful if the material used allows such conclusions. Thus the authors have not taken into consideration that the mere fact that repeated pregnancies predispose to diabetes may bias the material and by itself may give rise to a similar distribution of progeny. Furthermore, in all series there will be the tendency that th parents, one or both of whom have developed diabetes, on an average will be older than parents without diabetes, and as the net fecundity of the populations concerned, most of them of anglo-saxon origin, has been steadily decreasing ever since the change of the

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Table I Diabetes in twins

	All pairs			Juvenile		
	Total no.	Concordant	Discordant	Total no.	Concordant	Discordant
MZ-twins	53	32	21	6	6	0
DZ same-sex	76	10	66	5	0	5
DZ different-sex	82	12	70	7	2	5
Total	211	54	157	18	8	10

century this implies that the sibships with diabetic parents are older and therefore larger than sibships with two normal parents. Thus the material presented must be considered more or less inconclusive.

The points of view set forth in the following on the selective forces in diabetes are based on figures drawn from the Danish Twin Registry Details as to the collection and verification of medical data in this material have repeatedly been published elsewhere (3 4) This material now comprises around 7 000 pairs of twins both monozygous and dizygous, taken from the birth registers from the years 1870—1910 and followed up until the present time or until their death This twin material offers the advantage of constituting a closed population of 14 000 individuals with elaborately detailed medical histories. The total number of diabetics in this population will form a fully representative sample and with regard to all traits it is extraordinarily easy to draw comparisons with a comparative control series composed of individuals of the same time and place of birth, the same sex, the same social standard etc. as that of the proband series.

The first problem which may be answered by this twin material is

To what degree does the manifestation of diabetes mellitus depend on genetic factors?

This problem is elucidated by comparing the concordance rate in monozygous twins with the concordance rate in dizygous, same-sex twins. The whole series comprises a total of 304 diabetic probands, but a considerable number of these cannot reasonably be included in the calculations because the co-twin has died at an early age. As a more realistic approach to the theroretically correct concordance rate only such pairs have been taken into consideration where the healthy co-twin has reached the age of manifestation of diabetes in the proband. Table I gives the details of 211 pairs where this was the case. The concordance rate in monozygous twins is $\frac{32}{53} = 0.60$ in dizygous same-sex pairs $\frac{10}{76} = 0.13$ The difference is highly significant ($P < 0.001$) The formula

$$\frac{\text{Concordance rate in MZ twins} - \text{Concordance rate in DZ-twins}}{1 - \text{Concordance rate in DZ-twins}}$$

indicates to which degree the difference between two dizygous twin partners (and therefore also between two ordinary sibs) depends upon hereditary factors

with regard to the trait in question. For manifest diabetes the expression gives

$$\frac{0.60 - 0.13}{1.00 - 0.13} = 0.54$$

that is a practically equal influence of genetic and exogenous factors.

Fig. 1 shows a comparison between the concordance rates in monozygous and dizygous, same-sex twins with regard to some common disabilities. It appears that diabetes is distinguished by the highest concordance rate in monozygous twins combined with an appreciable difference between the concordance rate in monozygous and dizygous pairs.

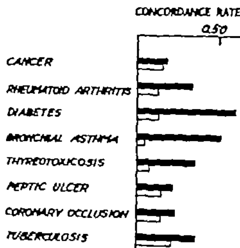


Fig. 1. Concordance rates in different clinical groups.
■ = MZ-twins.
□ = DZ-same-sex.

Then-Berg (7) has demonstrated in her twin-series from Germany that about two thirds of the apparently non-diabetic co-twins in her monozygous, discordant pairs had abnormal glucose tolerance tests in contrast to only one fourth in the dizygous, discordant pairs. This means that if the criteria for concordance are changed, so that pairs one of which has manifest diabetes, the other only diabetic glucose tolerance tests are considered concordant, the substitution with these figures in the formula will result in a considerably higher "heredity index". The interpretation must be that the glucose tolerance is chiefly determined by hereditary factors whereas the development of manifest diabetes in persons with an abnormal glucose tolerance is to a certain extent environmentally influenced.

It must be admitted, however, that the applicability of the above mentioned formula has been disputed, and without doubt the numerical result must be interpreted with caution. Regardless of the justifiability of using the formula the fact that hereditary factors play an important role in diabetes mellitus seems irreproachable. The traditional objection that the environments of monozygous co-twins are more alike than the environments of dizygous pairs so that their higher degree of similarity may be partly or totally environmentally determined is not valid: the affinity to a certain environmental background may just as well be considered a result of gene action, though not as direct, as may an inborn error of metabolism.

For a hereditary disease the incidence of diabetes is very high. According to Hansen (2) the morbid risk in a Scandinavian urban population is near 1 % at the age of 40 years and approximately 8 % at the age of 80 years, higher in females than in males. Under the assumption of recessive inheritance Steinberg and Wilder (6) estimated a gene frequency of the diabetic gene of 0.224, which means that 40 per cent of the population are carriers of either one or two diabetic genes.

Table II. Number of offspring in diabetic twins and their co-twins, MZ and DZ twins considered together

	Total no. examined	Total no. of offspring		No. of offspring per individual	
		Obs.	Expect.	Obs.	Expect.
Diabetic males	102	270	336	2.6	3.3
Diabetic females	128	405	365	3.2	2.8
All diabetic twins	230	675	701	2.9	3.0
Male co-twins of diabetic twins	116	278	258	2.4	2.2
Female co-twins of diabetic twins	121	284	271	2.3	2.2
Co-twins of diabetic twins	237	562	529	2.4	2.2

How may this high frequency of diabetic genes be explained?

When a gene is in equilibrium in the population (which is perhaps never the case) the total effects of negative selection must be balanced by contrary forces which tend to increase the frequency of the gene in question. Thus at a constant mutation rate a high gene frequency is the result of either a very low selection against the affected individuals or of the fact that the gene under certain circumstances, e.g. in heterozygotes, may imply a selective advantage.

The twin material, in which the number of offspring has been registered, may to a certain extent elucidate this question with regard to diabetes. Details are shown in table II which compares the observed number of offspring of diabetic twins and their co-twins with the number of offspring in control series. The controls have been selected among twin pairs of the same age, the same social standard, the same place of living (urban, provincial or rural) and the same zygosity as the probands of the group in question. As controls of the diabetics only such persons have been selected who were alive at the age of manifestation of diabetes in the proband.

It appears from the table that all differences are slight and insignificant. Contrary to Aschner and Post (1) the fecundity of the diabetic twins is found a little lower than in the controls, and this tendency is strengthened if the females, in whom repeated pregnancies may have a diabetogenic effect, are not included. After all it seems justified to conclude that the selection against diabetes is extremely slight.

The co-twins of the diabetics form a group in which a rather high percentage of gene carriers should be expected. Neither does any significant difference appear between this group and the controls, however, and the only conclusion allowed is that if the gene possesses a heterozygous advantage it is in any case trifling.

The most probable explanation of the high gene frequency in diabetes thus seems to be that the selection against the disease is not very active. This, however, is only true when all forms of diabetes are considered together. In juvenile diabetes the fertility must be considerably lower than the fertility of the general population, but this is nearly outweighed by a higher fertility in patients with diabetes of late manifestation. As appears from

Table III Relative fertility in groups of diabetic twins from different birth periods

Diabetic twin probands	Total no. in group	No. of offspring		Index of relative fertility
		Obs.	Expect.	
Males born 1870—89	58	171	177	0.97
Females born 1870—89	54	214	220	0.97
Total born 1870—89	112	385	397	0.97
Males born 1890—1910	44	99	159	0.62
Females born 1890—1910	74	191	145	1.31
Total born 1890—1910	118	290	304	0.95

table I, however the twins with juvenile diabetes in this material constitute too small a group to be dealt with separately

It must be borne in mind that these considerations concerning the gene frequency are valid only on the assumption that diabetes is due to a single or a few different genes. As shown by Post (5) no family study published so far refutes the theory of recessive inheritance of diabetes as a monomeric trait, but on the other hand the numerical relationship found may just as well be explained as the result of multifactorial inheritance.

The adaptiveness of the diabetic gene or genes will be varying under different environmental circumstances, and it must be suspected that such social factors as movements of the population from rural to urban districts, industrialization, falling birth rate and death rate, higher medical standard etc. will highly influence the selective forces working against diabetes.

Just as the development of the present social standard of the population has taken place during a rather short period of time, it must be expected that the selective value of the diabetes gene may be totally changed from one generation to the next. The introduction of insulin in the early twenties has been suspected to be the most important single factor by increasing the number of patients with juvenile diabetes in the reproductive age period. The next problem must therefore be to decide

To what degree has insulin influenced the gene equilibrium in diabetes

For this purpose the relative fertility of groups from different birth periods in the present material has been compared (table III) Certainly the relative fertility does not seem to have changed much when males and females are considered together but it should be noticed that the male fertility is much lower in the younger age group, whereas the opposite tendency predominates in the females. The latter may reflect the bias caused by the diabetogenic effect of repeated pregnancies which will be more pronounced in the younger age group because of the decreasing fertility with fewer children per marriage in the general population. To avoid this bias it is necessary only to consider the male fertility as a true expression of the selective value of the diabetes gene or genes. It is somewhat surprising that the changes here show just the opposite direction of what might

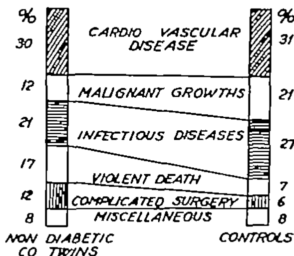


Fig 2. Cause of death in 113 non-diabetic co-twins compared with 482 controls.

be feared, the fertility of those diabetics born in the later period being significantly ($0.01 > P > 0.001$) lower than the fertility of those born in the early period in spite of the fact that the fertile period of the older group approximately 1890—1930 mainly falls before the era of insulin, whereas the fertile period of the younger group approximately 1910—1950 mainly falls after the introduction of insulin

The interpretation of these findings must be that the improved therapy has influenced only to a limited extent the total score of the selective forces in diabetes, in other words factors of which our knowledge is scarce or nil determine the genetic evolution of the disease. In this respect, also the twin material may be to a certain extent informative. The healthy co-twins of the diabetic probands constitute a group in which a considerable concentration of diabetic genes must be expected and in whom there is a possibility to study other potential effects of the gene, as far as the size of the material allows.

Fig 2 compares the causes of death of the non-diabetic co-twins of diabetic probands with those of a control series of non-diabetic twins. It turns out that there are considerable differences, most pronounced with regard to violent death and death from "complicated surgery" which comprises all deaths in connection with operations except those for malignant growths. The excess of deaths from these causes in the co-twins of diabetics is highly significant ($P < 0.001$) Deaths from malignant growths and infectious diseases, on the opposite, show a preponderance in the controls, the difference is not significant, however With regard to cardiovascular diseases and diseases collected under the heading "miscellaneous" the groups compared are approximately equal.

Because of the limited size of the material all conclusions must be drawn with caution. The conformity with regard to cardiovascular diseases may indicate that the predisposition to arteriosclerosis in diabetes is not the result of a direct gene effect, but is secondary to the disease. The lower number of cancer deaths may be an expression of a cancer protecting effect of the diabetes gene or may be due to chance.

The difference with regard to violent death and "complicated surgery" is more difficult to explain. It may of course, be due to chance, but certain circumstances suggest that a re-examination on a larger scale would be worth while. If the higher death rate from violent death in carriers of the diabetes gene holds true, the adaptive value of the gene would be expected to be lowered in such environments, where violent death is frequent. This will be the case in many primitive populations, where also the risk of diabetes is known to be low Thus, in the Eskimos of Greenland, where accidents under the hard living conditions

until the present time have been the main cause of death, the incidence of diabetes is very near zero.

Of course, hypotheses of this sort must be considered merely as speculative, until the findings have been confirmed in other materials. Furthermore several other explanations may be advanced with regard to the low incidence of diabetes in primitive populations. On the other hand the discrepancies between the causes of death in diabetes gene carriers and in the controls may be a reflection of a pleiotropic effect of the diabetes gene, i. e. the gene does not restrict itself to bring about diabetes, but also may influence other characters, each of which has a definite selective value. Somehow this may be one of the reasons why the final selective score of the diabetes gene carriers is decreasing at the same time as the therapeutic effectiveness is increasing

Summary and conclusions

In twin population comprising a total of 7,000 pairs 504 diabetics were found. A concordance rate of 32/53 = 0.60 in monozygous twins compares with a rate of 10/76 = 0.13 in dizygous, same-sex twins. The fertility of the diabetics is only slightly lower than that of the general population. For male diabetics born in the period 1870—1889 the relative fertility is 0.97 which differs significantly from that of male diabetics born in the period 1890—1910, which is 0.62 With regard to the causes of death non-diabetic carriers of the diabetic gene (or genes) show significant differences from the general population, especially with regard to deaths from accidents, including surgical complications.

The material substantiates the following conclusions Heredity is a most important etiological factor in diabetes. The high frequency of diabetes compared with most other genetic diseases is explained as a result of the very mild selection against the disease, especially of course in late diabetes. The introduction of insulin does not seem substantially to have influenced the selective value of the diabetes gene, on the contrary a lowering of the relative fertility has been recorded in the younger generation.

Acknowledgement

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The Peripheral Blood Flow in Intermittent Claudication

IV. The Significance of the Claudication Distance

By

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Pain originating during exercise is the leading symptom of coronary and peripheral artery disease, and the amount of exercise required to precipitate pain has been used as an index of the extent to which the blood supply is actually restricted.

Although this procedure is principally correct, it may be subjected to criticism because there are several other factors in addition to muscular exercise (8, 19, 22) which may affect the pain.

It was therefore of interest to examine the relationship between pain and restricted arterial circulation in order to find out to what extent the exercise tolerance is determined by the blood supply alone. Further this study aimed at answering the following questions: How far are the results of the exercise tolerance test influenced by the body weight, the rate of walking, the environmental temperature, the site of the obliterating lesion? How great is the spontaneous variation of the exercise tolerance at constant blood flow? How great is the blood flow response following exercise until muscular fatigue compared with that following exercise until unbearable pain?

Some of the above problems have been examined by others (3, 14, 16) but mostly in normal subjects.

Material

All patients studied suffered from intermittent claudication of the calf due to obliterative arteriosclerosis. None of them had other diseases.

The arteriographic examination revealed that the prevalent site of the arterial obstruction was in the superficial femoral artery. In the remaining patients the main lesion was located in the aorto-iliac or the popliteal-tibial arteries.

Methods

Experimental room, skin and muscle thermometry

Reference is made to previous papers.

Walking tolerance on the treadmill

The treadmill was constructed according to well-known models (4, 21). The range of speed was from 2 to 6 km/hour. The walking

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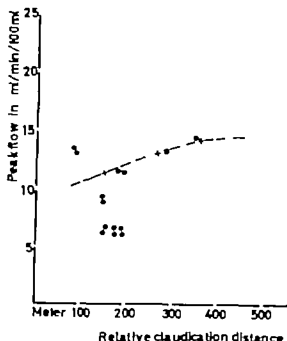


Fig 1 The relationship between the muscle blood flow of the calf and the relative claudication distance on a treadmill in intermittent claudication of the calf. Peak flow of the calf hyperemia following 1 min. of ischemic exercise

speed and the distance were recorded by an apparatus placed outside the sight of the patient The patients were instructed to describe their symptoms during walking The relative claudication distance was taken at the point where a sensation of tightness or initial pain occurred in the calf. The absolute claudication distance was taken at the point where the pain forced the patient to stop Unless otherwise stated the speed of the treadmill was kept at 3 km/hour and the room temperature at 30° C.

In the experiments on the effect of body weight the patients wore a bag on their shoulders. The bag was successively filled with loads of from 10 to 40 % of their body weight. Rest periods of about 40 min. were employed between each time the patients walked on the treadmill.

Ergography

The ergograph was essentially as that described by Goetz (6) An upright foot board was suspended in such a way that it moved around an imaginary axis passing through the ankle joint. When the foot piece was depressed, a weight was lifted 21 cm, the corresponding work being mainly done by the posterior calf muscles. The movement of the foot piece was automatically stopped at its lower endpoint, and simultaneously the patient relaxed his muscles. The descent of the weight thereafter drove the foot-board back into the upright position. Thus alternate contraction and relaxation of the calf muscles were obtained. The rate of ergography was signalled by means of a metronome. Usually a weight of 4.5 kg was employed at the ergograph.

The effect of weight was examined by keeping a constant rate of 60 contractions a minute (contr./min.) and by continuing the exercise until the pain forced the patient to stop In studying the blood flow the exercise was stopped after 1 min.

The effect of speed was evaluated by using a constant weight of 4.5 kg, and by continuing the exercise until unbearable pain. The blood flow responses were studied by keeping the amount of work made at a constant magnitude. Thirty contractions were made in 30, 60 and 90 sec. respectively the corresponding rates being 60, 30 and 20 contr./min.

When examining the exercise tolerance at the various room temperatures a weight of 7.5 kg was employed for the normal limbs and a weight of 4.5 kg for the ischemic ones. The ergography was carried out with the patient in the supine position.

Plethysmography

This was made as described in previous papers. Some definitions are repeated. The *peak flow* is the maximal hyperemia flow minus the blood flow at rest. *The excess flow* is the total hyperemia flow above the blood flow at rest.

The blood flow was recorded following exercise with occluded circulation (ischemic exercise) as well as with free circulation (free exercise) According to Grant (7) an increase of the blood flow above the resting level following local exercise of the forearm muscles, can be safely attributed to a change in the circulation through the forearm muscles. This is considered true also with respect to the calf circulation.

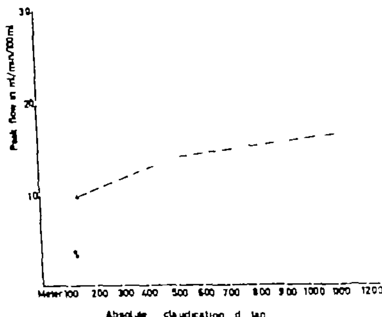


Fig. 2. The relationship between the muscle blood flow of the calf and the absolute claudication distance in intermittent claudication of the calf. Peak flow as in fig. 1

Table I. The effect upon the claudication distance of increasing the body weight by wearing loads of from 10 to 40 % of the latter. The body weight of the patients ranged from 60 to 82 kg with an average of 72 kg. Mean values from examination of ten patients with intermittent claudication of the calf

Body weight kg	Load %	Claudication distance	
		m	%
72	0	463	100
72	+ 10	389	84
72	+ 20	304	68
72	+ 30	233	50
72	+ 40	193	42

Ergography combined with plethysmography is technically rather difficult and is felt as an uncomfortable procedure by many patients. The range of weights and rates used throughout the experiments had therefore to be restricted, and the duration of the exercise had to be shorter than originally intended.

Table II. The effect of increasing the load of the ergograph on the number of calf muscle contractions until claudication of the calf. Mean values from examination of ten limbs with intermittent claudication of the calf

Load of the ergograph		Muscle contractions	
kg	%	No.	%
2.5	100	293	100
5.0	200	91	31
7.5	300	64	21

Results

Exercise tolerance and blood supply

There is a certain relationship between the claudication distance and the calf blood flow but the wide range of the values prohibits any safe use of the distance as an index of the blood flow (fig. 1 and 2). These findings indicate that in addition to the blood flow there

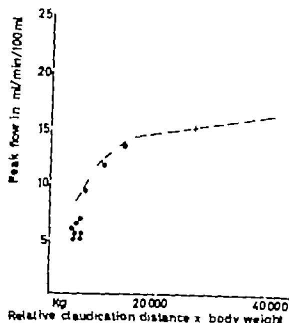


Fig 3 The relationship between the muscle blood flow of the calf and the production of the relative claudication distance and the body weight. Peak flow as in fig 1

are other factors at work in determining the walking distance. A study of some of these factors is presented below

The effect of weight

As expected the body weight plays a main role in limiting the distance (table I) The percentage reduction of the walking tolerance is greater than the corresponding increase of the body weight.

The effect of the weight in local exercise of the calf muscles is significant, but comparably less (table II)

When corrected for the influence of the body weight, the relationship between walking tolerance and flow becomes improved (fig 3 and 4)

The blood flow response to exercise with different weights reveals the typical pattern, which separates the normal from the pathological circulation (table III and IV) In response to increasing weights at the ergograph the peak flows of the ischemic limbs increase less than normal. Their excess flows are very much above normal and increase in approximate proportion to the weight.

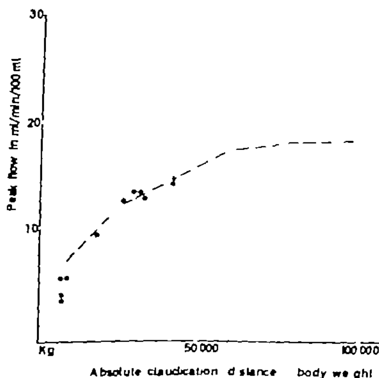


Fig 4 The relationship between the muscle blood flow of the calf and the production of the absolute claudication distance and the body weight. Peak flow as in fig 1

Table III The effect of increasing the load of the ergograph upon the peak flow of calf hyperemia following 1 min. of free exercise. Mean values from examination of five normal limbs and six limbs with intermittent claudication of the calf. Blood flow in ml/min/100 ml tissue (ml)

Load of the ergograph		Peak flow			
		Normal limbs		Ischemic limbs	
kg	%	ml	%	ml	%
2.5	100	9.7	100	8.9	100
5.0	200	14.5	150	9.9	110
7.5	300	17.1	173	12.5	137

Table IV The effect of increasing the load of the ergograph upon the excess blood flow of the calf. From the same experiments as table III

Load of the ergograph		Excess flow			
		Normal limbs		Ischemic flow	
kg		ml	%	ml	%
2.5	100	3.7	100	19.0	100
5.0	200	15.1	408	27.1	143
7.5	300	20.3	549	62.8	331

Table V The effect of different speed upon the walking performance in ten patients with intermittent claudication. Mean values are given

Speed of the treadmill		Claudication			
		Distance		Time	
km/hr	%	m	%	sec	%
2	100	612	100	1,102	100
3	150	390	64	468	43
4	200	287	47	258	23

Table VI The effect of different speed of the ergograph upon the calf muscle contractions until claudication. Mean values from examination of ten limbs with intermittent claudication

Speed of the ergograph		Claudication			
		Contractions		Time	
Contr./min	%	No.	%	sec	%
20	100	103	100	316	100
30	150	92	88	184	58
40	200	73	71	113	36
60	300	68	63	68	22

The rapid increase of the normal excess flows can not be explained. It may be an over-shooting phenomenon. However it may as well mean that just in the range of work made in this experiment, the normal flow through the muscles during the exercise rapidly becomes inadequate. The result is a correspondingly rapid increase of the post-exercise flow

The effect of speed

The rate of walking is also important in determining the claudication distance (table V) although it seems to be of less significance than the weight. This is the case provided the comparison is based upon the percentage expression of the results. A calculation reveals that the absolute amount of work made at the different magnitudes of body weight is greater than the work made at the different rates. These considerations also hold true regarding the effect of varying speeds at ergography (table VI)

A peculiar effect of speed merits attention. The results suggest that the time of claudication is much more affected by the speed than is the distance.

The blood flow responses to exercise at different rates show no main difference from those to different weights (table VII and VIII) The flow values are less all over probably as a result of the different amounts of work performed. It is difficult to make an accurate calculation of this difference. But when a tentative

Table VII The effect of increasing the speed of the ergograph upon the peak flow of the calf hyperemia following free exercise The amount of work performed at the various speeds was kept constant (see methods) Mean values from examination of five normal limbs and five limbs with intermittent claudication of the calf Blood flow in ml/min/100 ml tissue (ml)

Speed of the ergograph		Peak flow			
		Normal limbs		Ischemic limbs	
Contr/min	%	ml	%	ml	%
20	100	8.2	100	7 0	100
30	150	10.6	130	8.9	127
60	300	12.4	150	10.3	147

Table VIII The effect of different speed of the ergograph upon the excess blood flow of the calf From the same experiments as in table VII

Speed of the ergograph		Excess flow			
		Normal limbs		Ischemic limbs	
Contr./min	%	ml	%	ml	%
20	100	3.5	100	7.0	100
30	150	4.6	130	10.4	150
60	300	9.0	260	15.8	225

calculation is made, and the flow responses are tentatively corrected for the different amount of work, the flow responses to various speeds are still less than the responses to various weights.

The net result of the experiments on the effect of speed is that peak flows do not keep pace with the increase of rate, whereas the excess flows show an approximately proportional rise

The effect of environmental temperature

No appreciable difference of the muscular working capacity could be demonstrated at the different room temperatures (table IX) This finding is supported by results of examination of the blood flow following exercise at the same ambient temperatures (table X)

The blood flow response to exercise until fatigue or until absolute claudication of the calf muscles

When exercise with arrested circulation is employed the vascular reserve capacity becomes apparent (table XI) Due to the dissimilar amount of exercise the absolute figures of the two kinds of limbs should not be compared, but attention paid to the great difference between the normal flow responses at the two extremes of exercise In comparison the corresponding difference between the ischemic flow responses is negligible.

Table IX The effect of different environmental temperatures upon the number of muscle contractions made until claudication The absolute figures of the normal and ischemic limbs should not be compared (see methods) Mean values from examination of five normal limbs and ten limbs with intermittent claudication of the calf Skin temp of the 1st toe Muscle temp. of the posterior calf muscles Temperature readings made just prior to the commencement of the exercise

Room temp. (C°)	No. of contractions		Skin temp. (C°)		Muscle temp. (C°)	
	Normals	Patients	Normals	Patients	Normals	Patients
10	101	166	15.5	14.3	34.8	32.6
20	113	189	24.0	21.0	35.3	32.1
30	116	173	33.8	31 0	35.8	32.5

Table X The peak flow of calf hyperaemia following 1 min of free exercise and the calf blood flow at rest at different environmental temperatures. Mean values from examination of three normal and six ischemic limbs Blood flow in ml/min/100 ml tissue

Room temp. (C°)	Peak flow	Blood flow at rest
10	13.2	2.1
20	13.5	3.1
30	13.5	4.0

Table XI The calf blood flow following ischemic exercise of two lengths, the one producing fatigue and the other unbearable claudication of the calf To obtain comparable data the normal limbs were exercised for about the same lengths of time namely, e 60 sec and 90 sec respectively Mean values from examination of two normal and four ischemic limbs Blood flow in ml/min/100 ml tissue

Calf muscle response	Peak flow		Excess flow	
	Normal limbs	Ischemic limbs	Normal limbs	Ischemic limbs
Fatigue	29.3	11.2	48.7	34.5
Claudication	42.5	12.5	84.1	47.9

Table XII The same experiments as in table XI employing free instead of ischemic exercise The normal limbs were exercised for 90 sec and 150 sec respectively

Calf muscle response	Peak flow		Excess flow	
	Normal limbs	Ischemic limbs	Normal limbs	Ischemic limbs
Fatigue	27.0	9.0	17.4	27.5
Claudication	33.7	8.9	38.1	35.3

Table XIII The influence of different site of the obliterative lesion upon the claudication distance The latter is expressed as the product of absolute claudication distance and body weight. Peak flow following 1 min of ischemic exercise Mean values from examination of fifty-two limbs with intermittent claudication of the calf and, in which no appreciable over-lapping was present between the different sites

Site of main lesion	Claudication distance	Peak flow
Aorto-iliac arteries	36,080	16.4
Femoral superf. artery	48,300	14.1
Popliteal-tibial arteries	31,350	10.3

Under normal circumstances the skeletal muscles are working with free circulation (table XII) If the experiments are repeated with free circulation, an important conclusion becomes warranted. There is practically nothing to gain in terms of blood flow by extending the exercise beyond the point at which fatigue occurs in the calf muscle. If the excruciating pain of the absolute claudication is considered, it seems mandatory to stop the exercise at the sensation of muscular fatigue.

The effect of the site of the obliterative lesion

As far as the blood flow through the calf muscles is concerned, aorto-iliac obstructions cause a relatively greater reduction of the walking tolerance than those which are situated at lower levels (table XIII) This finding probably influences the earlier demonstrated diagrams on the relationship between flow and distance. Some of the high flow values with corresponding short distances belong to limbs with aorto-iliac stenoses.

The spontaneous variability of the claudication distance

The examination of this subject was carried out in constant environmental temperature, at the same time of day t the same rate of walking and with the same amount of clothes. The plethysmo-

Table XIV The spontaneous variation of the relative claudication distance in 10 patients with intermittent claudication of the calf

Months				G	S. d.	V. c.
1	2	3	4			
68	84	50	80	70.5	15.3	21.6
130	170	147	160	152.0	17.3	11.4
70	80	90	80	80.0	8.2	1.2
55	90	90	180	104.0	53.5	51.4
70	70	80	70	72.5	5.0	6.9
277	480	340	280	344.0	95.0	27.6
110	110	140	200	140.0	42.5	30.4
140	170	120	140	142.5	20.6	14.5
315	240	390	425	342.5	62.8	18.3
80	90	130	135	109.0	27.8	25.5
Mean var (indiv.) 131.5	158.4	157.7	175.0	157.7	32.7	20.9%
Monthly var (group) 131.5	158.4	157.7	175.0	155.7	18.0	11.5%

Table XV The spontaneous variation of the absolute claudication distance in 10 patients with intermittent claudication of the calf

Months				G.	S. d.	V. c.
1	2	3	4			
110	128	130	157	131.3	19.4	14.8
250	350	336	330	316.5	45.1	14.3
140	220	300	500	290.0	154.3	53.3
125	300	300	500	306.3	117.8	38.5
150	160	200	160	167.5	22.2	13.3
300	540	450	390	420.0	101.0	24.1
225	270	500	650	411.0	193.0	43.5
235	330	275	650	372.5	189.0	50.8
485	290	740	740	566.0	218.3	38.6
220	250	300	310	270.0	42.5	15.7
Mean var (indiv.) 224	283.8	353.1	438.7	325.1	111.0	31.2
Monthly var (group) 224.0	283.8	353.1	438.7	324.9	72.0	22.3*

graphically obtained blood flows were not appreciably altered from time to time. Yet the variation of the walking distance was great (table XIV and XV). The mean individual variation as well as the variation of the whole group was less for the relative than for the absolute claudication distance.

Comments

The unsatisfactory relationship which has been demonstrated between walking distance and blood supply is of interest in three respects. Firstly it shows the necessity of being extremely careful in using the exercise tolerance as an index of the actual blood supply. Secondly it suggests that other aspects of the obliterative disease than the blood circulation need study. In other words, there is place for all the indirect methods such as thermometry, oscillometry of different kinds, ergography and others. Thirdly it indicates the possibility of improving the walking tolerance by other measures than those aimed at increasing the blood flow alone. Unfortunately such additional therapy is often neglected.

It is not easy to transfer the results obtained by means of ergography on supine patients into the state of walking. The effect of weight and rate is more pronounced in the erect posture. The results of previous studies made by ischemic exercise of normal forearm muscles (14, 16) are therefore difficult to apply in intermittent claudication of the calf. In contrast the data of the present

report on the effect of body weight and speed upon the walking tolerance are ready for practical use. More so as the patients studied were of medium body weight and the rates employed were of an order which usually are used by such patients. The experiments indicate the quantitative improvement of exercise tolerance, which may result from weight reduction and slower walking speed. Noteworthy also is the fact that the walking speed exerts a stronger influence upon the claudication time than upon the claudication distance. A reduction of the rate will thus cause a larger increase of the time than of the distance. In slowing down the speed the patients will therefore have a feeling of having walked for a longer distance than is virtually the case.

In principle the blood flow responses to various weights and rates were similar The peak flows of the ischemic limbs were less and increased less rapidly than normal. Their excess flows were abnormally large and increased at approximate proportions to weight and rate. Although obtained by free exercise the latter results are in accordance with those obtained by ischemic exercise of normal forearms (16)

The flow studies reveal some essential features of the peripheral circulation. The blood flow in rhythmic exercise of the skeletal muscles is normally arrested during the muscle contractions (1) Between these contractions the blood is streaming rapidly into the dilated muscular arteries. If the amount of blood passing through the muscle during work is too small to meet the demand, the latter has to be met when the exercise is over This becomes apparent as an increase of the excess flow This event is clearly illustrated by the normal blood flow response to weight in this report. It has also been clearly shown by Black (3) in his studies of the normal calf flow in walking and running In ischemic limbs this normal circulatory response occurs at a much lower amount of work than in normal limbs. The rigid and obstructed arteries of the ischemic limb do not supply the muscles with enough blood during exercise. Consequently the demand has to be paid after the cessation of exercise. The resulting large excess flows are also demonstrated by the flow studies of this report.

Some times the amount of work is of such an order that the peak flows are nearly identical in the normal and the ischemic limbs (table III) However they can be easily distinguished from each other by assessing the excess flows (table IV)

Another point is also worth mentioning. At the lowest amount of weight employed (table III) the peak flows are not very different. The same amount of work is made at the same rate by all the limbs. Consequently the excess flows ought to be of approximately the same order When this is not the case, it is due to the decreased velocity of the blood in the ischemic limb The reduced velocity does not appear in the peak flows as used in this report, but becomes apparent as delayed peak flows in the post-exercise hyperemias. This has been demonstrated by Shepherd (20) and will be outlined in another paper of this series.

A majority of patients state that their walking distance becomes reduced at low environmental temperatures. According to the present report such an effect of the ambient temperature can be excluded. This is keeping with the fact that the skeletal muscle blood flow at rest is unaffected by body heating (2, 5) and

with the fact that the vasoconstrictor nerve supply to the skeletal muscle is sparse.

The post-exercise hyperemia is considered to have a beneficial effect upon the development of collaterals The greater this hyperemia, the greater probably the collateral promoting effect If the hyperemia following exercise until absolute claudication is considerably larger than that following exercise to less extents, the patient must be advised to walk briskly until the pain forces him to stop According to the present report such advice is wrong By extending the exercise beyond the point of muscular fatigue there occurs an increase of the duration of the hyperemia whereas the peak flow is unchanged The gain in terms of blood flow by extending the walking to absolute claudication is small and out of proportion to the very uncomfortable pain. The best advice to give the average patient with intermittent claudication is to take frequent walks until muscular fatigue of the calf. Whether exercise to real claudication in addition to being useless also may have harmful effects, can not be answered in this report However it has been observed clinically that such exercise may produce a permanent deterioration of the peripheral circulation (18)

That the degree of arteriolar dilatation is the same at the point of fatigue as at the point of unbearable pain rules out powerful vasodilatation as the cause of pain This finding is more in favour of the old metabolite theory (14) Unfortunately the present knowledge of this subject is inadequate (12)

By means of ergography Lindstrom (15) showed that the aorto-iliac stenosis reduced the exercise tolerance comparably more than stenoses at lower levels. His finding is supported by this report. Such information is of consequence for the previously demonstrated diagrams on the relationship between flow and distance. Some of the high flow values with corresponding short distances belong to cases with aorto-iliac stenoses.

For the practical use of the walking test a thorough knowledge of its spontaneous variation is necessary Various reports have revealed variation of coefficients from below 10 % to about 28 %, (9 13) In this connection the controlled study made by Hess (10) is very impressive. He noted a 100 % increase of the claudication time irrespective of whether the patients had been treated with a vasodilator drug or with an inert tablet. However his diagram shows that the walking time of the two groups improved at the same rate prior to as during treatment. Such great spontaneous improvements have also been noted by others (17) This is by no means surprising in view of the many factors which may affect muscular pain, and in view of the possible effect of training It has been suggested that training may enhance the neutralizing action of blood towards lactic acid and thereby allow an improvement of the walking tolerance without any corresponding increase of the blood flow (11)

The present report demonstrates that the walking distance varies considerably from month to month at a relatively constant blood flow The variation coefficients are considerably less for the relative than for the absolute distance This could be expected as the will of the patients affects the point of absolute claudication more than the point of the relative claudication. Moreover it is obvious that valid conclusions can hardly be based upon observation of single patients.

The best procedure is to examine a suitable group of patients and employ walking until muscular fatigue as the exercise test. By means of such a procedure the great spontaneous variation can be circumvented.

Summary

The exercise tolerance of limbs with intermittent claudication was examined by means of a treadmill and an ergograph. The calf blood following exercise was estimated by means of venous occlusion plethysmography

The walking tolerance was found to be an unsatisfactory index of the actual blood supply to the calf.

A successive increase of the body weight reduced the walking tolerance by more than equal amounts. A successive increase of the rate reduced the walking distance by approximately equal amounts, whereas the reduction of the walking time was much greater Similar results were obtained by means of ergography although the effect of weight and rate was less than in the erect patient.

The calf blood flow was studied following exercise with various weights and at various rates. Characteristic features of the normal and pathological circulation were demonstrated.

The exercise tolerance of normal and diseased limbs was examined at room temperatures of 10 20 and 30 centigrade. Likewise the blood flow following exercise was estimated at these temperatures. No significant effects of the ambient temperature were noted either on the exercise tolerance or on the blood flow

When the exercise was extended from the point of relative to the point of absolute claudication the blood flow response was remarkably little altered. The peak flow remained at the same level, while the excess flow showed a small increase. There is very little to gain in terms of blood flow by extending the exercise beyond the point of muscular fatigue.

Obstructions situated at the aorto-iliac level exerted a greater reduction of the walking tolerance than did obstructions at lower levels of the main artery

The spontaneous variation of the claudication distance was examined in the course of four months in limbs with a constant blood flow The relative distance revealed a variation coefficient of 20.9 % for the single patient and of 11.5 % for a group of ten patients. The corresponding figures for the absolute distance were 31.2 % and 22.3 %.

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Follow up Studies on an Unselected Ten-year Material of 360 Patients with Liver Cirrhosis in one Community

By

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The natural history of liver cirrhosis has been the subject of extensive surveys based on large series. The present investigation was considered legitimate for two reasons. Firstly the material, which is the largest on record in Scandinavia, shows the behaviour and nature of the disease in this part of the world. Secondly the material consists of patients from the town of Malmo (210,000 Inhabitants) which is served by one hospital only and is thus unique for epidemiological studies.

Material

The investigation, which was started in Jan. 1961 is based on all the 360 cases of liver cirrhosis admitted to Malmo General Hospital during 10-year period (1951—1960). Of these patients, 292 died during this period. Data were collected from the necropsy protocols and from the hospital record sheets of the departments of internal medicine, surgery and infectious diseases, as well as of Malmö Sjukhem, department for convalescents and patients with chronic diseases.

Only cases with a firm diagnosis were accepted. I 280 the diagnosis was based on autopsy findings, in 45 on liver biopsy and in the remaining the diagnosis was regarded as firm only if the patient had shown signs of liver injury such as bilirubinaemia, electrophoretic abnormalities or increased BSP retention, as well as signs of portal hypertension such as splenomegaly oesophageal arices, ascites or increased wedged hepatic venous pressure.

The series was grouped according to cause of the disease from an aetiological point of view Objections may of course, be raised against grouping in this way but this also pplies to other classification systems such as the widely used morphological classification with the groups portal or Laennec s cirrhosis, postnecrotic cirrhosis and biliary cirrhosis. A drawback of morphological classification is that aetiologically different types of cirrhosis may exhibit the same final pathological pictur and that it is, generally speaking, difficult to classify a clinical series morphologically (12) even though some authors claim that it is possible on the basis of biopsy findings. Our six groups were cryptogenic, alcoholic, biliary posthepatitic, cardiac cirrhosis and haemochromatosis. The cryptogenic group embraced cases of unknown origin. T the group alcoholic cirrhosis were assigned those patients who were known to be heavy drinkers (considerable daily consumption of

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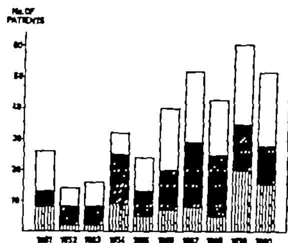


Fig. 1. Liver cirrhosis in Malmö 1951—1960. 360 patients (214 males and 146 females).

☐ = Cryptogenic.
▨ = Alcoholic.
▥ = Others.

strong (40—45 %) spirits, often up to one litre a day). The posthepatitic group consisted of those with a history of hepatitis as the only explanation available. The other groups do not require special comment.

It is difficult to draw a sharp line of distinction between the groups. In the cryptogenic group, for example, nothing was known about the drinking habits of some of the males, and it is possible that some of them were heavy drinkers. In the alcoholic group 7 patients had previously had hepatitis, but there was reason to assume that the major cause of the cirrhosis was the abuse of alcohol, for the interval between the spell of jaundice and the onset of the symptoms of cirrhosis was usually very long (fig. 3) and the consumption of alcohol was heavy.

Incidence

The number of cases discovered tended to increase (fig. 1 and table I). During the 10-year period signs of liver cirrhosis were seen in 3.3 % of all the 8,279 patients autopsied.

Comments

The frequency with which liver cirrhosis is found at autopsy varies from series to series (table II) and probably with hospital facilities available, the frequency of autopsy and probably with any particular interest in liver diseases. The incidence found — 3.3 per cent — may be taken as representative of the frequency of the disease in a random population of 210,000 in a town where, at least in recent years, about two thirds of all who died were examined *post mortem*.

It is difficult to explain the increase in the frequency of liver cirrhosis during the last 10 years. It is true that the population of the town has increased from about 190 000 to 230 000 during this period and that the hospital facilities have improved at approximately the same rate, but this will hardly explain the difference, particularly since the department of pathology noted not only an increase in the total number but also in the percentage of cases of cirrhosis (table I).

Until 1955 the purchase of spirits in Sweden was rationed. That year however restrictions were withdrawn. This resulted in an increased consumption of alcohol and a consequent increase in the frequency of admissions for delirium tremens in the department of psychiatry — in 1960 16 % of all male admissions were because of this diagnosis as against 8 % in 1951. The number of cases probably due to alcoholism has also tended to increase — possible more since 1955 — but still more the incidence of the non-alcoholic types of liver cirrhosis.

During the years 1946 to 1949 Malmö witnessed an epidemic of hepatitis. In 1948, at the culmination of the infection, as many as 0.17 % of the total population had jaundice — in Sweden infectious hepatitis is reportable. But this epidemic can hardly be held responsible for the increase in the frequency of liver cir-

Table I Liver cirrhosis in Malmö 1951—1960 Autopsy series

	Year										
	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	Total
No. of cases autopsied	564	648	648	671	758	803	941	938	1,054	1,184	8,279
With liver cirrhosis.	8	8	9	19	16	24	36	43	56	52	273
With liver cirrhosis (%)	1.4	1.2	1.4	2.8	2.1	3.0	3.8	4.6	5.3	4.4	3.3

Table II Incidence of liver cirrhosis found at autopsy in different countries

Country	Authors	No. of autopsies	Cirrhosis (%)
Austria	Holzner et al. (18)	24,008	1.8
Chile	Aranda-Cruz et al. (2)	400	8.5
Germany	Langer Hoever (23)	40,126	2.3
Switzerland	Riva, Werner (30)	5,482	6.1
USA	Hall et al. (15)	16,600	4.4
USA	Kirshbaum, Shore (20)	12,267	2.8
Other countries.	Ratnoff, Patek (28)	—	0.7—10.1
Sweden	Present	8,279	3.3

rhosis in the late 1950s. The increased mortality from cirrhosis noted in Denmark after a corresponding epidemic occurred soon after the epidemic had reached its height (4). Moreover the present series includes only 5 cases with jaundice during the years 1946—1949

The wider use of refined diagnostic methods during the 1950s enabled the clinical diagnosis of otherwise doubtful cases, and this may at least partly explain the increase in the frequency of cirrhosis, but it will not explain the increase in the frequency found post mortem

Remarks on the aetiological groups

Cryptogenic and alcoholic cirrhosis

The cryptogenic and alcoholic groups were the largest and represented 43 % and 34 respectively of the entire series (fig 2) As in most series on record, the alcoholic group consisted mainly of males, and the cryptogenic group mainly though not so markedly of females. As mentioned previously the cryptogenic group probably includes some cases of alcoholic cirrhosis, which, if they had been assigned to the group to which they really belong would have accentuated the difference in the frequency of the disease with sex still more. Further clinical differences found between these two groups and described later justify the grouping on aetiological grounds.

Biliary cirrhosis

Only 4 of the 39 patients belonging to this group are still alive. One male and 4 female cases were conceived as the primary biliary type all had complained of itching and they all had profound jaundice (serum bilirubin 4.3, 5.5 9 4

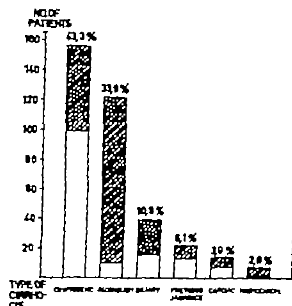


Fig 2 360 cases of liver cirrhosis in Malmö 1951—1960

▨ — Males.
□ — Females

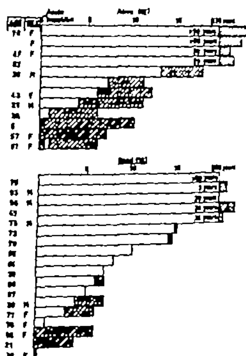


Fig 3. Clinical course of liver cirrhosis in 30 patients with previous jaundice.

□ — Latent period
▨ — Manifest liver cirrhosis.
A — Alcoholism.
B — Biliary Cirrhosis (Extrahepat. obstr.)

25 and 60 mg/100 ml) and high alkaline phosphatase values (14 27 41 44 and 45 U Buch & Buch) Only one patient had hypercholesterolaemia — 470 mg/100 ml The values noted in the remaining 4 were below 260 mg/100 ml and none had xanthomatosis. Of those who had extrahepatic obstruction, 18 had occlusive cancer and 15 stone in the common bile duct The patients with stone had not been missed by the surgeon they had either refused operation or were poor risks.

Posthepatitic cirrhosis[1]

All the 30 cases in which the cirrhosis had been antedated by jaundice and in which hepatitis was believed to be the cause are included in fig 3 Seven of these cases — marked A in the figure — were, in our opinion alcoholics, so that in these it is very difficult to say under which heading they should really be listed But in 5 of these cases histological examination had shown fine nodular cirrhosis of Laennec's type. Two patients, clearly of biliary type (B) were also deducted this left 21 cases classified here as posthepatitic cirrhosis. In 12 of these cases the patients have in the meantime died, and only 5 of them showed changes fitting in with a diagnosis of postnecrotic cirrhosis. The remaining 7 had fine nodular or Laennec's cirrhosis, but in none of them was the liver enlarged.

Of this series then 8 / had a spell of jaundice in their history and in 6 ° the liver had presumably been damaged by infectious hepatitis. It is, of course, debatable whether the infectious hepatitis had been the causative agent in all of these 6 Such a causal relationship can only be concluded with certainty in those

[1] Previous jaundice in fig. 2.

Table III Frequency of different types of cirrhosis in various series

Country	Authors	No. of cases	Cryptogenic (%)	Alcoholic (%)	Previous jaundice (%)	"Posthepatitic" (%)
Austria	Holzner et al. (18)	439	41	16	21	—
Chile	Armas-Cruz et al. (2)	208	—	78	25	—
England	Sherlock (32)	100	43	18	33	—
Finland	Halonen, Sahala (16)	134	—	20	8	—
Finland	Adlercreutz, Sandberg (1)	226	32	29	23	15
France	Godlewski (14)	4,358	—	82	—	1
Germany	Kalk (19)	137	44	8	—	36
Switzerland	Riva, Weber (30)	65	—	65	—	5.4
USA	Douglas, Snell (8)	444	18.6	64	3.6	—
Sweden	Present	360	43	34	8	6

cases in which the cirrhosis was secondary to histologically verified acute hepatitis without any symptomfree interval. The frequency of different types of cirrhosis in different countries is given in table III. The frequencies differ widely and it is possible that these differences were due to differences in the diagnostic criteria used. Previous jaundice does not necessarily mean that the patient had had virus hepatitis, for it may have been the initial clinical manifestation of liver cirrhosis. It would appear that hepatitis seldom gives rise to liver cirrhosis. Large numbers of soldiers who had had hepatitis during World War II were re-examined several years later and the frequency of liver cirrhosis was not found to be higher than in a control series (26, 34). The value of such an examination of young men is, however, limited by the fact that the epidemic of hepatitis in Denmark (4) during the 1940s was followed by an increased mortality almost exclusively among females. However, MacDonald and Mallory (25) believe the risk of cirrhosis following hepatitis is only 0.7 %. Summing up, it appears that the relationship between infectious hepatitis and liver cirrhosis is obscure and in the present paper the term posthepatitic is to be understood in its temporal rather than in its aetiological sense.

Cardiac cirrhosis

The cardiac group consisted of 7 males and 7 females, all with long-standing heart failure. Five had organic heart defects of rheumatic origin, 6 had arteriosclerosis, 2 had constrictive pericarditis, and 1 amyloidosis of the heart.

Haemochromatosis

The 8 patients with haemochromatosis included one woman, 80 years old. One of the patients was an alcoholic. Six had manifest diabetes and 4 showed signs of hypogonadism. Six had died before the end of the present period of investigation.

Sex

Of the 360 cases, 214 were males and 146 females, thus a ratio of roughly 1.5 : 1. The sex distribution of those who had died was about the same as that of the entire material.

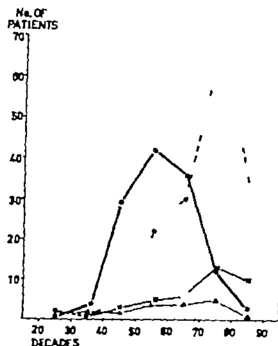


Fig. 4. Age in 339 patients with different types of liver cirrhosis.

○- - -○ = Cryptogenic.
○——○ = Alcoholic.
▲——▲ = Posthepatitic.
×——× = Biliary.

The relatively high frequency of females was noteworthy. Popper and Shaffner (27) collected 2 576 cases from the literature and found a sex distribution of 2.3 : 1. Many of the series on which these figures are based consisted mainly of cases with portal cirrhosis with a consequent overrepresentation of male alcoholics. Ratios of 3.2 : 1 (23) and 1.7 : 1 (18) have also been reported in unselected series. It is thus clear that the frequency of females in the Malmö series was relatively high.

Age distribution

The age distributions in the 4 largest groups are given in fig. 4, from which it is apparent that especially in the cryptogenic group the patients were very old. Thus together with the figures noted for the sex distribution warrants the conclusion that the alcoholic group consisted mainly of males, aged 40 to 70 years (mean age 57) while the cryptogenic group was dominated by females, aged 60 to 90 years (mean age 70). Old patients were also most common in the biliary group, which may be due largely to the high frequency of old patients with occlusive cancer of the biliary tract.

Many authors have reported the same or a somewhat lower mean age for portal cirrhosis (2, 13, 15, 28) than was found in our alcoholic group, which, according to morphological classification, corresponded best to Laennec's cirrhosis. As to the cryptogenic cases, the patients in the clinical series of Summerskill et al. (33) were younger than in the alcoholic group. The marked preponderance of old people — mainly females — in our series with cryptogenic cirrhosis in Malmö is unique and difficult to explain. It should perhaps be pointed out that our myeloma patients also belong to higher age groups than anywhere else.

Symptoms and signs

The initial symptoms were generally fatigue, anorexia, loss of bodyweight, increased girth and oedema of the legs as well as diffuse abdominal symptoms, usually meteorism and diarrhoea. Epigastric pain or pain under the right costal arch was also relatively common and sometimes severe. The clinical symptoms are summarized in table IV a. The table also includes the subclinical cases, in which the diagnosis was not known until after necropsy. This explains why the frequencies of the symptoms in the table are somewhat lower than in a series consisting of cases with a clearcut

Table IV Physical symptoms and signs

	Entire material	Cryptogenic	Alcoholic	Biliary	Post hepatitic	Cardiac	Haemochromatosis
A. Patients with liver cirrhosis							
	(360)	(155)	(122)	(39)	(22)	(14)	(8)
	%	%	%	%	%		
Hepatomegaly	42	23	63	44	13	12/14	3/8
Ascites.	38	33	47	28	32	8/14	0
Oesophageal varices	34	35	46	3	23	1/14	4/8
Vascular spiders	23	12	43	5	27	0	0
Splenomegaly	22	22	28	5	36	2/14	0
Gastrointestinal haemorrhage.	19	15	34	5	9	1/14	0
Coma	15	15	14	15	32	0	0
B. Patients with clinical diagnosis of liver cirrhosis							
	(219)	(72)	(106)	(13)	(18)	(2)	(8)
	%	%	%				
Hepatomegaly	54	38	66	10/13	3/18	2/2	5/8
Ascites	52	56	54	8/13	6/18	2/2	0
Oesophageal varices.	47	48	54	3/13	4/18	1/2	4/8
Vascular spiders	36	22	51	2/13	8/18	0	0
Splenomegaly	34	36	37	3/13	7/18	1/2	0
Gastrointestinal haemorrhage	28	19	41	2/13	2/18	0	0
Coma	27	26	26	5/13	3/18	0	0

clinical diagnosis, and exemplified by table IV b, in which the figures include only those cases in which the diagnosis was known before autopsy

Hepatomegaly

As expected (2, 6, 11 20) palpable enlargement of the liver was noted mainly among alcoholics. If clinically diffuse cases be excluded (table IV b) the liver appeared t be enlarged in 38 of the 72 cryptogenic cases. This frequency is much higher than that (9 %) given by Summerskill et al. (33) for this type of cirrhosis. But since palpation is not a very reliable method for estimating the size of the liver (28) the weights of the livers noted in the autopsy protocols were also analysed (see below)

Ascites

Clinically demonstrable ascites was most common among the alcoholics. The frequency found for the cryptogenic group was also high (3,33)

Oesophageal varices

Signs of portal hypertension in the form of oesophageal varices had been demonstrated roentgenologically or *post mortem* in half of the alcoholics and in one third of the cryptogenic group Summerskill et al. (33) found oesophageal varices to be somewhat more common in the cryptogenic than in the alcoholic group Baggenstoss (3) reported a much higher frequency of varices in alcoholic cirrhosis than in posthepatitic cirrhosis. In this respect then, the posthepatitic re

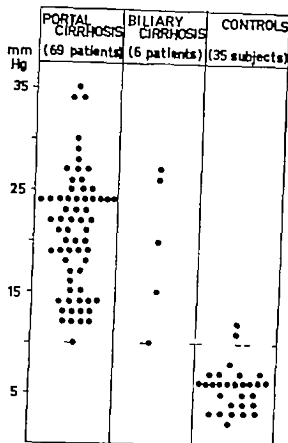


Fig 5 Wedged hepatic venous pressure in 75 cases of liver cirrhosis.

sembled the cryptogenic group in the present series.

Haemorrhage

Bleeding from oesophageal varices was twice as common in the alcoholic group as in the cryptogenic group namely in one third of the cases which is a very high frequency for this complication (2, 8 17 28 33)

Splenomegaly

Splenomegaly was demonstrated clinically roentgenologically or *post mortem* in 1 of every 5 patients, irrespective of the type of cirrhosis. In the biliary group splenomegaly as well as other signs of portal hypertension was rare. Of the patients in table IV b splenomegaly was seen in 1 out of every 3 patients, which is also a low frequency for this complication (15 28 29)

Hepatic coma

Hepatic coma was equally common in all groups. It is a serious complication and only 1 of every 3 who had had it, survived. All 20 however died later 16 of them in a recurrent coma, 3 from haemorrhage, and 1 from pneumonia. Eleven patients died soon after the initial spell of coma. The remaining 9 survived on the average 10 months (range 2 months to 3 years) Therapy consisted — at least in the latter years — of protein restriction, broad spectrum antibiotics — particularly neomycin — abundant fluid and glucose. Today the prognosis of liver hepatic coma is probably much brighter than that suggested by the figures available from the entire last decade.

Increased wedged hepatic venous pressure

The wedged hepatic venous pressure was recorded in 75 cases (fig 5) For simplicity the cryptogenic, alcoholic and posthepatitic groups are pooled, since they did not differ from one another in this respect. The pressure recorded in each patient is plotted in the diagram, from which it is clear that almost all of the recordings exceeded the normal upper limit (10 mm Hg) In 2 cases of biliary cirrhosis the pressures only bordered the upper limit — it is known that the symptoms of portal hypertension develop slower in this type of cirrhosis. It may thus be concluded that a normal wedged venous pressure argues strongly against a diagnosis of liver cirrhosis, provided of course that the disease is not complicated by portal thrombosis. This complication was observed in 5 % of the autopsy series and most of these had

primary or secondary carcinoma of the liver

The pressure recordings given in fig 5 refer to patients with a firm clinical diagnosis. The corresponding table IV b shows that the symptoms of hypertension — with the exception of gastrointestinal bleeding, which was particularly common among the alcoholics — was largely the same in the alcoholic as in the cryptogenic group. The dominance of symptoms of portal hypertension observed among patients with cryptogenic cirrhosis in the series of Summerskill et al. (33) did not occur in the present material. One might expect the resistance offered to the portal flow by a small shrunken liver to differ from that offered by a large fatty liver But no correlation could be demonstrated between liver weight and the wedged hepatic venous pressure. Neither could any correlation be found between the portal pressure, as judged from the weight of the spleen, and the weight of the liver in 184 patients at autopsy

Hypersplenism

Hypersplenism is not uncommon in the presence of splenomegaly It is known that haemolytic anaemia sometimes occurs in liver cirrhosis. This complication is, however of complex origin and will not be discussed here. The relation between leukopenia and size of the spleen is noteworthy Leukopenia — here to be understood as values below 4,000 leukocytes/mm — was noted in 20 / of 340 patients studied. Of 264 patients without demonstrable enlargement of the spleen, 11 % had leukopenia, as against 54 of 39 patients with a palpable spleen.

As to thrombocytopenia (less than 100,000/mm) the corresponding figures

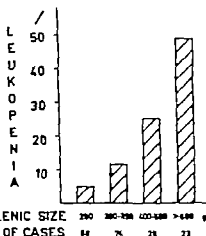


Fig. 6. Relationship between splenic size and leukopenia — leukocyte count <4,000 mm³ — in 183 cases of liver cirrhosis.

were 2 % and 37 % respectively but the number of patients studied was not large enough to warrant any valid conclusion.

The weight of the spleen was noted in 183 of the autopsy protocols. The patients were grouped according to this weight and the percentage with leukopenia in each group was calculated. The correlation found between the number of leukocytes and the size of the spleen is apparent from fig 6 which illustrates in greater detail the findings described in the preceding paragraph.

Biochemical findings

It is difficult to give characteristic values for each patient because many of them had been admitted to hospital more than once and examined on several occasions during each spell. In the present analysis we chose the most abnormal value noted during the last spell of the patient in hospital.

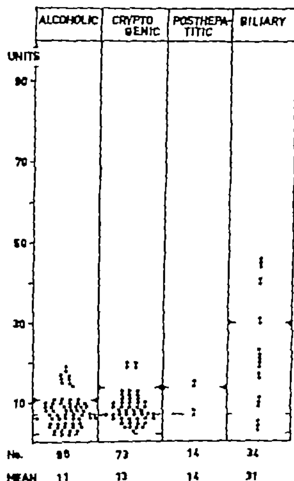


Fig 7 Alkaline phosphatases in 211 patients with liver cirrhosis.

Serum bilirubin

In 360 patients the degree of bilirubinaemia was examined It was noteworthy that normal values were found in as many as 25 % of these patients A normal finding was particularly common in the cryptogenic and posthepatitic groups while, as expected the values were high in the biliary group

Bromsulphalein test

The retention of bromsulphalein was found to be normal in only 3 of 108 patients examined. In these 3 cases the clinical picture was somewhat diffuse but the diagnosis had been confirmed by biopsy It should be mentioned that the BSP retention was studied only in those cases in which there was reason to suspect cirrhosis on clinical grounds. These patients thus represented a selected group.

Alkaline phosphatase

The results of the alkaline phosphatase determinations are given in fig 7 The normal range (2—7 units) is enclosed by interrupted lines. The figure shows that a moderate increase of these values was common. The mean value noted for the biliary group was, as expected more than moderately increased.

Cholesterol

Of 96 patients studied the serum cholesterol was normal (150—250 mg/100 ml) in 65 % In 20 % it was below 150 mg/100 ml, and of these 19 patients 17 have died. A low serum cholesterol level is not characteristic of liver cirrhosis, but low values are prognostically unfavourable.

Thymol turbidity test

Of 239 cases examined normal values were found in as many as 40 %. This high percentage was due largely to the fact that in half of the alcoholic group the values were not increased. In this respect the alcoholic and biliary types of cirrhosis differ from the cryptogenic and posthepatitic. This difference is still more conspicuous if those cases not diagnosed before autopsy be excluded the value for the cryptogenic group then being still higher

Serum albumin

The impaired synthesis of albumin in the damaged liver is reflected in fig 8. The normal limits (4.2—5.3 g/100 ml)

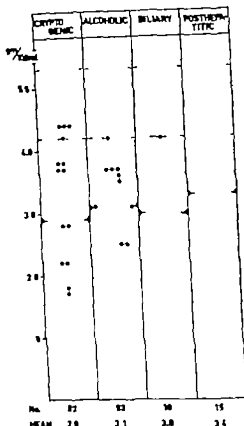


Fig. 8. Serum albumin in 208 patients with liver cirrhosis.



Fig. 9. γ-globulin in 208 patients with liver cirrhosis.

are given in the same way as in fig 7 and the mean value for each group is given t the bottom It is clear from the figure that the values were normal in only few of the cases and that no significant d fferences were found between the means of the groups.

Serum γ-globulin

The γ-globulin 'alues showed the opposit tendency (fig 9) In most of the cases the 'alues exceeded the normal upper limit (1 10 g/100 ml)

I some patients the values noted were very high, particularly in the lupoid" group. (These cases have been described in a previous paper (21)) It consisted of 11 patients, most of whom were assigned here to the cryptogenic group and a few to the posthepatitic group They were characterised not only by hepatocellular damage and high γ-globulin values, but also by systemic lupus-like manifestations from various organs, some times a positive LE-cell phenomenon pathological serological reactions and often a marked infiltration of plasma cells and lymphocytes of the liver In addition they also responded favourably to steroid therapy

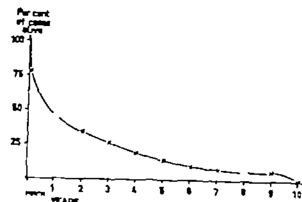


Fig 10. Survival after diagnosis in 163 patients with liver cirrhosis.

Co-existing diseases

Diabetes

Diabetes mellitus deserves mentioning It occurred in 15 % of the entire series, or in 13 % if the cases of haemachromatosis be excluded Only 5 8 % of 4 167 patients autopsied during 1957—1960 had a clinical diagnosis of diabetes. It was particularly common in the alcoholic and biliary groups (16 % and 18 % respectively) This series differs from many others (13 14 28) by its higher frequency of diabetes. It is not possible to offer any satisfactory explanation for the difference in this respect between the above mentioned autopsy series and the group of cirrhosis

Peptic ulcer

Of the patients included in the present material 11 % had peptic ulcer No substantial difference was found in this respect between the alcoholic and cryptogenic groups.

Gallstones

Excluding the biliary group gallstones were found in 24 % of the 256 patients autopsied The corresponding figure for 4 167 autopsies during 1957—1960 was 18 % The slight difference can probably be explained by the dominance of males in the cirrhosis group (5)

Syphilis

Syphilis or a positive Wassermann-test had been noted in the records of 5 %. Of these 18 patients, 13 had reported that they had had the disease and had been treated a few decades previously and 4 of them had positive serological reactions. Two of 5 who denied ever having had syphilis had positive TPI test and the remaining 3 had such strongly positive reactions that they were regarded as specific. In none of the above-mentioned 18 patients, mainly alcoholics was the disease regarded as active or as being *per se* of aetiological importance.

Rheumatoid arthritis

Chronic polyarthritis was noted in 10 patients with cryptogenic cirrhosis and 3 in the other groups. This corresponds to 3 6 % of the entire material. If those with lupoid cirrhosis, which is known to be often accompanied by rheumatic symptoms, be excluded the figures will be 2.3 % The frequency of 3.6 % is in good accord with that found by Lane et al. (22) in an epidemiological study

Prognosis

The fact that only 68 of the 360 patients were still alive shows that the prognosis of the disease is gloomy This is also borne out by the curve in fig 10 which shows the duration of survival after diagnosis of the fatal cases. Less than half survived 1 year one fourth 3 years and only one tenth 6 years. In the evaluation of these figures the high average age of the patients must, however also be considered.

Table V Cause of death in 287 patients with liver cirrhosis

	Cryptogenic (%)	Alcoholic (%)	Biliary (%)	Post hepatitic (%)	Cardiac (%)	Haemochromatosis (%)	Total (%)
Coma	18	20	17	50	—	17	19
Haematemesis	7	25	3	7	—	—	11
Cancer	11	18	—	—	—	17	11
Other	64	37	80	43	100	66	59

The percentage of deaths within 1 year is in accord with investigations of the prognosis by previous authors (8 11 28, 29) The difference, from a prognostic point of view between the aetiological groups reported by Summerskill et al. (33) (50 % of the alcoholic group and 29 % of the cryptogenic group dead within 5 years) could not be confirmed in the present material.

Cause of death

Coma and haemorrhage

Of the causes of death a special note was made of coma, oesophageal bleeding and primary carcinoma of the liver (table V) One fifth died in coma — no substantial difference was found between the groups in this respect Every tenth patient died from oesophageal bleeding Of these 33 patients, 21 succumbed to the first bleeding and 9 to the second.

It is known that hepatic coma is a much more common cause of death than oesophageal bleeding (5 7 10, 17 28, 29) Many authors have reported higher percentages of deaths from hepatic coma than that found in the present material. The discrepancies in the literature between the frequencies given for hepatic coma as the direct cause of death are probably due to the difficulty in deciding whether coma in the final stage is of hepatic or other origin.

Primary carcinoma of the liver

Cases of primary carcinoma of the liver are of certain interest. The condition was seen in 31 (11 %) of the autopsy series. This is a fairly moderate value compared with the varying and sometimes very high values reported in different countries (27 32) The equal frequency of carcinoma of the liver in the two large aetiological groups does not lend support to the assumption (9 24) that the tendency for carcinoma of the liver to develop varies with the type of cirrhosis. The ratio of males to females was 4 1 and is in accord with that found by previous workers in this field (32) The mean age was 67 years. The final cause of death was coma in 18 cases and oesophageal bleeding in 7

Other causes"

The patients who had died from other causes" included several in whom cirrhosis had been a contributory cause of death e.g the patient with severe liver failure who died from bronchopneumonia, i.e a disease which could otherwise surely have been controlled. In other words, the number of deaths from liver cirrhosis was

in reality greater than that indicated by coma, oesophageal bleeding and carcinoma together

Autopsy findings

The results of the *post mortem* examinations will probably be the subject of a later paper therefore only a few gross findings will be commented upon here.

Liver

It might be mentioned that as in several other respects, no substantial difference was found between the post hepatitic and cryptogenic groups. Assuming 1,500 g as the normal weight of the liver the organ was enlarged in only one third of the cryptogenic group as against four fifths in the alcoholic group As to the cases in the cryptogenic group with liver weights of more than 2 000 g all 9 had primary or secondary carcinoma of the liver Of the 6 alcoholic cases with an extremely low liver weight one, the only woman in the group showed signs of postnecrotic cirrhosis, as did one of the men who a few decades previously had had an attack of jaundice.

In this respect the cryptogenic group resembled the posthepatitic or postnecrotic group in other series on record (3 29 33) In the evaluation of the size of the liver the patient's age must be taken into account. The mean age of the alcoholics was 57 years, that of the cryptogenic group 70 years. The physiological decrease in the weight of the liver between the ages 57 and 70 is, however only about 100 g (31) The difference in weight of the liver between the two groups can therefore not be ascribed entirely to the difference in age.

As to the gross appearance of the liver it was simply described as coarsely nodular (excluding cases of primary and secondary carcinoma of the liver) in 4 % of the 50 cases of alcoholic cirrhosis and in 4 % of the 23 cases of biliary cirrhosis against 26 % of the 78 cryptogenic and 36 % of the 11 posthepatitic cases.

Spleen

The weight of the spleen *post mortem* is probably not an accurate measure of the weight of the organ during life. The weights noted in 175 cases (89 cryptogenic, 60 alcoholic and 26 biliary) were, however analysed. The mean weight in the biliary group (282 g) was found to be lower than in the other groups (cryptogenic 363 g alcoholic 375 g) In the alcoholics the spleen was of normal size or only moderately enlarged (200—400 g in 60 %) while in the cryptogenic group it was normal or moderately enlarged in 27 % and in 40 % it weighed less than 200 g The mean weights in both groups were however similar because the organ was extremely large in a few cases in the cryptogenic group Most of the small spleens in this group were found in the very high age classes.

Cases diagnosed at autopsy

In 141 cases, *i.e.* about one third of the entire material, the diagnosis was not confirmed (26) or even suspected (115) before autopsy These cases will be described together with a pathologist in a separate paper and compared with the entire material.

Summary

A retrospective analysis of several aspects of the natural history of liver cirrhosis is given on the basis of all 360 cases of the disease diagnosed during a 10-year period (1951—1960) in a town hospital serving a population of 210,000 inhabitants.

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Urinary Excretion of Vitamin B_6 and Folic Acid in Achlorhydria and after Partial Gastrectomy

By

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It has earlier been observed by us that the urinary excretion of thiamine, riboflavin, pantothenic acid and biotine is reduced in achlorhydria, whereas no definite difference was observed in the excretion of the biologically active metabolites of nicotinic acid (2, 9) After partial gastrectomy the excretion of the above mentioned vitamins B is clearly influenced by whether or not achlorhydria resulted from the gastrectomy In the former case the excretion was usually still more reduced than in simple achlorhydria, but if acid secretion was preserved the excretion was near the level of the excretion of control subjects (9)

The object of the present work was to supplement the above mentioned studies by observation of the excretion of vitamin B_6 and folic acid in achlorhydria and after partial gastrectomy

We have found in the literature no reports on vitamin B_6 metabolism in achlorhydria and after partial gastrectomy Neither have, to our knowledge, any studies been carried out on the basal urinary excretion of folic acid in these conditions.

In a series of 10 patients with untreated pernicious anemia the folic acid activity in the serum was normal in 9 patients and elevated in one patient (7) The urinary excretion of folic acid after an oral dose of this vitamin is normal in pernicious anemia (1 6)

Six patients who had undergone partial gastrectomy 0—9 years previously had a normal blood level of folic acid (8) None of the 20 patients with intestinal malabsorption following gastric operation were found to have a disturbed folic acid absorption (5)

Material and methods

The determinations were carried out microbiologically Saccharomyces carlsbergensis was used in the determination of vitamin B_6 (3) and Streptococcus faecalis in that of folic acid (4)

The series consisted of a total of 103 test subjects. The control group was comprised of 46 hospital patients, none of whom had a renal, hepatic, intestinal or other severe disease that could be assumed to influence the metabolism of the studied vitamins. In the control series the vitamin B_6 excretion was determined in 34 and the folic acid ex-

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From the Departments of Medicine and Medical Microbiology University of Turku, Finland

Urinary Excretion of Vitamin B_6 and Folic Acid in Achlorhydria and after Partial Gastrectomy

By

Pekka Brummer and Toivo Markkanen

It has earlier been observed by us that the urinary excretion of thiamine, riboflavin, pantothenic acid and biotine is reduced in achlorhydria, whereas no definite difference was observed in the excretion of the biologically active metabolites of nicotinic acid (2, 9). After partial gastrectomy the excretion of the above mentioned vitamins B is clearly influenced by whether or not achlorhydria resulted from the gastrectomy. In the former case the excretion was usually still more reduced than in simple achlorhydria, but if acid secretion was preserved the excretion was near the level of the excretion of control subjects (9).

The object of the present work was to supplement the above mentioned studies by observation of the excretion of vitamin B_6 and folic acid in achlorhydria and after partial gastrectomy.

We have found in the literature no reports on vitamin B_6 metabolism in achlorhydria and after partial gastrectomy. Neither have, to our knowledge, any studies been carried out on the basal urinary excretion of folic acid in these conditions.

In a series of 10 patients with untreated pernicious anemia the folic acid activity in the serum was normal in 9 patients and elevated in one patient (7). The urinary excretion of folic acid after an oral dose of this vitamin is normal in pernicious anemia (1, 6).

Six patients who had undergone partial gastrectomy 0—9 years previously had a normal blood level of folic acid (8). None of the 20 patients with intestinal malabsorption following gastric operation were found to have a disturbed folic acid absorption (5).

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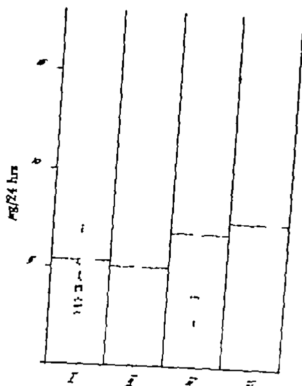


Fig 1 Excretion of folic acid. I Controls II Test subjects with achlorhydria III Gastrectomized patients with acid gastric secretion, IV Gastrectomized patients with achlorhydria.

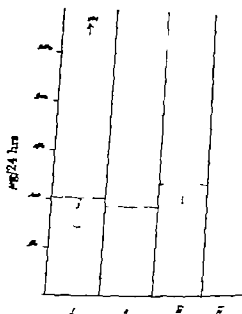


Fig 2 Excretion of vitamin B_6. I. Controls II Test subjects with achlorhydria III. Gastrectomized patients with acid gastric secretion IV Gastrectomized patients with achlorhydria.

cretion in 38 subjects. The test series included 22 patients with histamine proved *achlorhydria*, 10 of whom had pernicious anemia. The folic acid excretion was observed in all these test subjects and the vitamin B_6 excretion in 14 of them. There were 35 subjects who had previously undergone partial gastrectomy for peptic ulcer Acid gastric secretion was preserved in 23 of these patients. The vitamin B_6 excretion was determined in 25 and the folic acid secretion in 31 of the subjects in the postgastrectomy group

Results

The urinary excretions of vitamin B_6 and folic acid in the different groups is shown in figs. 1 and 2. The values are the means of 2-day excretion.

Individual variations from person to person in the daily excretion of both vitamins were fairly marked. Nevertheless, no clear differences were seen in the mean excretion of vitamin B_6 or of folic acid in the different groups. The two low folic acid excretion values (0 0 and 0 8 µg) seen in the achlorhydria group occurred in patients with pernicious anemia. The mean excretion of folic acid in test subjects with simple achlohydria was 5.8 µg 24 hrs.

On the basis of the present results it is evident that achlorhydria does not produce disturbances in the B_6 and folic acid metabolisms similar to those seen in the metabolism of thiamine, riboflavin pantothenic acid and biotine. The reason for this difference cannot be definitely explained in the light of our present knowledge. It seems probable that the disturbances seen in achlorhydria in the metabolism of the vitamins of the B group

mentioned in the beginning of this paper are due chiefly to changes in the balance between intestinal vitamin synthesis and absorption. It is possible that under these conditions the increased synthesis of some vitamins B may compensate the possibly decreased absorption.

Summary

The authors have observed the urinary excretion of vitamin B_6 and folic acid in achlorhydria and after partial gastrectomy. No difference from normal values was observed.

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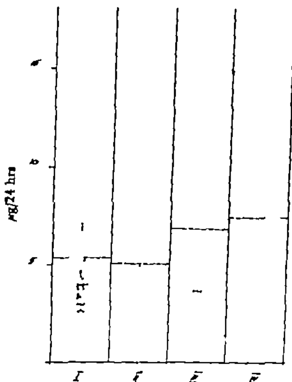


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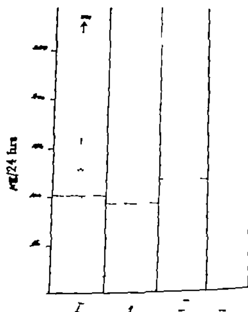


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Influence of Desferrioxamine on the Renal Excretion of Iron

Preliminary Report

By

Jørgen Boye Nielsen

It is generally recognized that the human body has little capacity to excrete iron. Only minimal quantities are excreted with the bile and urine, by desquamation of cells from the skin and mucous membranes, and by the growth of hair and nails. This loss hardly exceeds 0.5—1 mg daily. If the body is supplied with iron in a quantity exceeding the daily loss, the result will be an accumulation of iron in the tissues, and large accumulations give rise to the syndrome of haemochromatosis.

Out of the daily loss of 0.5—1 mg renal excretion makes up only about 0.05—0.10 mg (1, 2, 3, 4) despite the fact that the load on the kidneys is about 1 g iron in the transferrin bound form per 24 hours. Even in nephrotic states the renal loss of iron is slight, amounting to 0.5—1.0 mg per 24 hours, depending upon the proteinuria (1).

In an effort to increase the renal excretion of iron, various complex-forming substances have been used. Administration of BAL (dimercapol) to normal subjects does not increase the renal excretion of iron (5, 6) while a maximum excretion of 2.0 mg/24 hours has been observed in a patient suffering from transfusion haemosiderosis (6). EDTA gives only a very slight increase in the excretion in normals (7, 8, 9) while in patients with haemochromatosis a maximum excretion of 6 mg daily has been obtained (7).

It is of considerable interest, therefore that now a substance is available which appears to be able to increase the renal excretion to a greater extent. Desferrioxamine, which the substance has been called so far, belongs to the siderochromes, i. e. substances which with ferric iron form reddish-brown complex compounds. Most siderochromes are produced by microorganisms and are divisible into 2 main groups, sideromycins which are iron-containing antibiotics, and the sideroamines. Desferrioxamine which is a sideroamine, is a slightly basic, colourless, crystalline substance 100 parts of which can bind a maximum

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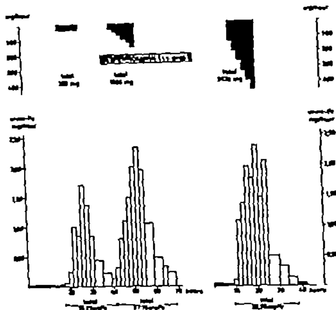


Fig. 2. Iron excretion after continuous i. v. infusions of deferrioxamine.

for autoradiographic localization. Fig 4 shows at the top the strip obtained by electrophoresis of the radioactive solution of ferrioxamine. The lack of staining must mean that the solution contains no protein. Just below this strip, on the extreme right, there is the blackening of the X-ray film caused by the radioactive solution of ferrioxamine. The fact that the blackening appears on the right of the site of application must mean that at the pH used the ferrioxamine is positively charged. Thereafter the figure shows the electrophoresis of plasma to which had been added radioactive iron, and below the blackening reveals that, as might be expected, the radioactive iron is situated at the position of the transferrin in the β_1-globulin. In the next row electrophoresis and autoradiography of non-radioactive plasma to which had been added radioactive ferrioxamine are shown. The blackening reveals that all the radioactive iron is still located at the site of the ferrioxamine while there is no blackening at the site of transferrin. At the bottom, there are the strips representing radioactive plasma to which had been added deferrioxamine. Here, there is distinct blackening at the site of transferrin and, in addition, incipient blackening at the site of ferrioxamine. Judging by this method, then, it seems as

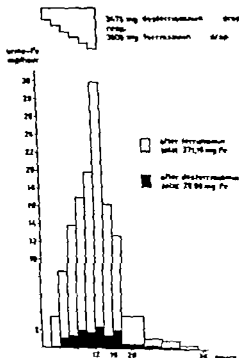


Fig. 3. Iron excretion after continuous i. v. infusions of deferrioxamine and ferrioxamine respectively.

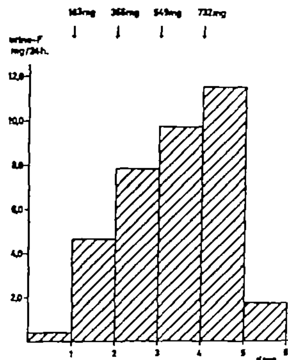


Fig 1 Iron excretion after i. v injections of desferrioxamine.

of 9 3 parts trivalent iron w/w The resulting ferric complex is called ferri oxamine Desferrioxamine has a molecular weight of 561 Its formula is $C_{25}H_{48}N_6O_8$ and in the form of the hydrochloride it is freely soluble in water (10) Desferrioxamine appears to be, at any rate to some extent absorbed in the gastrointestinal tract, whereas the ferric complex ferrioxamine is hardly absorbed at all (11) Accordingly it seems possible to block the intestinal absorption of iron by oral administration of desferri oxamine.

Experiments

As is apparent from fig 1 the renal excretion of iron is considerably increased by intravenous injection of desferrioxamine The figure shows also that the effect upon the iron excretion appears to decrease with increasing doses of desferrioxamine Lastly the effect is largely confined to the first 24 hours.

Furthermore, the effect upon the renal iron excretion of continuous intravenous infusion of desferrioxamine was investigated in a patient with confirmed haemochromatosis (fig 2) Continuous infusion of a total of 366 mg within 10 hours gave an iron excretion about twice that of the same dosage administered in the form of a single intravenous injection.

In order to investigate whether there is an upper limit to the amount of iron which may be excreted through the kidneys per time unit under the influence of intravenous desferrioxamine, the same patient was treated with a continuous, intravenous infusion of increasing doses of desferrioxamine. As is evident from fig 2 it was not possible to increase the renal excretion beyond 2.5 mg iron/hour in this patient despite further administration of considerable quantities of desferrioxamine.

To exclude the possibility that the upper limit depends on the renal excretion capacity, the same patient received a continuous intravenous infusion of increasing quantities of ferrioxamine (= desferrioxamine saturated with trivalent iron) in doses corresponding to those used in the desferrioxamine infusion. Fig 3 shows that now the renal excretion of iron increased considerably reaching about 30 mg/hour 84 of the iron administered in the infusion of ferrioxamine being recovered in the urine. In other words, the limit does not appear to be a question of renal capacity

Since the renal excretion of iron during the infusions of desferrioxamine exceeds the patient s normal plasma iron turnover during the period concerned at least part of the excreted iron must have been mobilized primarily from sites other than the plasma.

To investigate whether desferrioxamine is able to remove iron bound to transferrin, the following *in vitro* experiment was carried out, using 4 different solutions, viz. (a) a 2 % solution of desferrioxamine, (b) a radioactive solution of ferrioxamine prepared by incubating the solution of desferrioxamine with Fe^{59} (c) plasma from a normal subject, and (d) plasma to which was added a tracer dose of Fe^{59} These solutions and combinations thereof were subjected to electrophoretic separation on paper After drying the individual paper strips were cut longitudinally One half was stained in the usual way while the other half was placed on an X-ray film

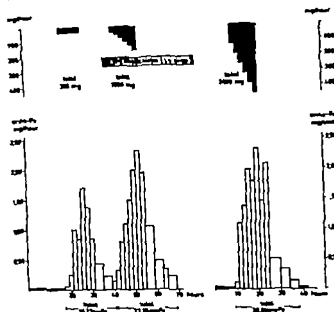


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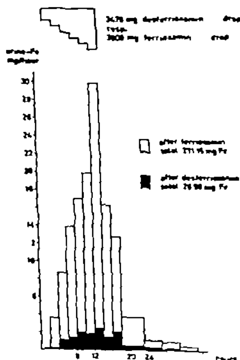


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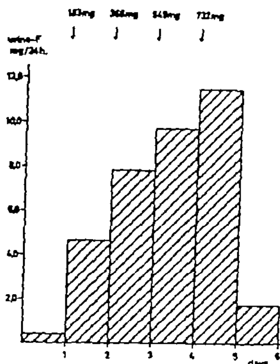


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Discussion

Desferrioxamine appears to be the strongest iron-binding chelate known so far. By intravenous administration the renal excretion could be increased to a maximum of 2.5 mg/hour. The fact that the excretion cannot be increased beyond this limit, despite further doses of desferrioxamine does not appear to depend on the renal excretion capacity. From the limited data, it is reasonable to assume therefore, that this upper limit of renal iron excretion of 2.5 mg/hour indicates the rate at which the iron depots may be mobilized. 2.5 mg/hour corresponds to 60 mg/24 hours, equalling the amount of iron required for the production of about 120 ml blood. This corresponds approximately to the clinical experience that in treating patients with haemochromatosis by phlebotomy it is impossible to draw 500 ml blood more often than every 5th—6th day without causing a decrease in haemoglobin. Moreover one might speculate that the rate of mobilizing the iron depots may be a factor limiting the haemoglobin production in the bone marrow.

According to the present experiments it seems likely that desferrioxamine is able to remove iron bound to transferrin. Whether desferrioxamine can take up iron direct from ferritin and haemosiderin, or whether preceding binding to transferrin is a necessary intermediate stage, has not yet been decided.

The practical application of desferrioxamine must be primarily in conditions involving abnormally increased iron depots. In haemochromatosis the renal excretion of iron may be maintained at about 50 mg for 24 hours by three daily intramuscular injections (12). Desferrioxamine may be imagined to acquire particular importance in the treatment of transfusion haemosiderosis, e.g. in aplastic and haemolytic anaemia where treatment by phlebotomy is out of the question. Lastly it may be expected that desferrioxamine will prove of value in the treatment of acute oral iron poisoning.

Summary

A new chelate forming substance desferrioxamine, is discussed. It appears to possess an exceptionally pronounced iron-binding ability. *In vitro* experiments showed the iron-binding capacity of desferrioxamine to exceed that of transferrin. Intravenous administration to patients with haemochromatosis induces a considerable increase in the renal excretion of iron. The maximum excretion was found to be 2.5 mg iron/hour. The upper limit does not depend on the renal excretion capacity but appears to represent the maximum rate of mobilizing the iron depots. On the other hand administration of desferrioxamine does not seem to cause any distinct increase in the renal excretion of calcium. Lastly the expected practical applications of desferrioxamine are mentioned.

Acknowledgements

Thanks are due to Dr Sv. Solvsten Sørensen, Isotope Department, Rigshospitalet, for having aided in planning and performing the *in vitro* experiments and to Dr J. Lorensen, Ciba Ltd., Copenhagen, for kindly supplying the desferrioxamine.

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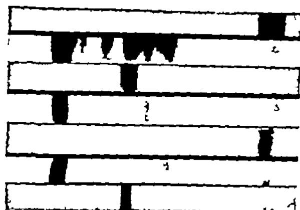


Fig 4 Autoradiograms of Fe^{59} after paper electrophoresis. For details see text.

if transferrin is unable to remove iron bound to desferrioxamine while desferrioxamine appears to be able to remove to some extent, iron bound to transferrin. A later complex constant determination (10) gave $K = 10^{30.7}$ for Fe^{+++} desferrioxamine. By way of comparison it may be mentioned that $K = 10^{27}$ and 10^{29} respectively for Fe^{++} transferrin and $K = 10^{25}$ for Fe^{+++} EDTA.

Another question which arises is whether desferrioxamine is capable of increasing the excretion of metals other than iron. However the determination of the relevant complex constants has not yet been completed.

To ascertain whether the excretion of calcium might be increased by the infusions of desferrioxamine, the renal excretion of calcium was also determined. There is perhaps (fig. 5) a moderate increase in the calcium excretion, the amounts excreted being 340 mg and 245 mg respectively in the two experimental periods, or 290 mg and 190 mg/24 hours. Since the patient was not at any time on a fixed low-calcium diet, these values cannot be considered definitely abnormal.

During the desferrioxamine infusions the serum calcium values were also determined (fig 5) but the results are not consistent. During the first experiment there was a gradual fall of serum calcium from 9.5 to 8.5 mg/100 ml, while in the second experiment with infusion of considerable greater quantities of desferrioxamine, the serum calcium concentration remained within the normal range. To explain this discrepancy it should be noted that while in the latter experiment the serum calcium was determined by X-ray spectrometry an EDTA titration was used in the former experiment. Thus, an interference between the desferrioxamine and the Ca-EDTA complex is possible which might have caused too low serum calcium values.

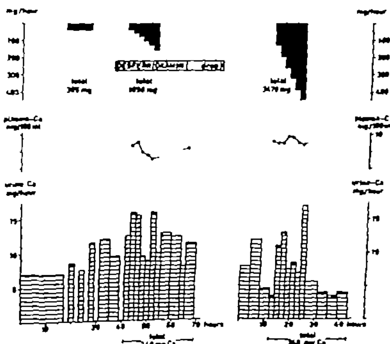


Fig 5. Calcium excretion and serum calcium concentration after continuous i.v. infusion of desferrioxamine.

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The Effect of X-ray Treatment on Leukocyte Alkaline Phosphatase in Cancer Patients

By

C. Wasastjerna, B. Jeglinsky and C. E. Nylund

Alkaline phosphatase is cytochemically demonstrable in neutrophilic polymorphonuclear cells of the peripheral blood. The amount of leukocyte alkaline phosphatase (LAP) varies appreciably from case to case in both normal and pathological states. There is general agreement that in most cases of chronic myelocytic leukemia (6 8, 11 12, 15 19 21) the amount is decreased, and that an increase is often noted during remission after X-ray therapy or treatment with radiomimetic drugs (19 23). Moloney and Lange (14) found decreased LAP activity in preleukemic conditions caused by irradiation.

Ionizing radiation produces changes in the enzyme activity of radiosensitive cells (3, 4). The LAP activity varies from cell to cell even under normal conditions, and is very sensitive to such influences as infections and stress (11 19 22). Although it thus seems likely that the LAP would respond to cellular damage caused by X-irradiation, there is no general agreement on the effect of X-rays on phosphatases. A number of research groups have studied the effect of whole-body irradiation on adenosintriphosphatase activity in animal tissues. The activity as measured by biochemical methods, was found to increase after X-irradiation in liver homogenates (2) in spleen homogenates (10) and in bone marrow and leukocyte suspensions (17). On the other hand Ackerman et al. (1) and Ellinger and Strike (5) found by histochemical means that there were decreased amounts of alkaline phosphatase in lymph nodes and the spleen of animals after irradiation, whereas Ludewig and Chanutin (9) noted no effect whatever on the enzyme activity in rat liver homogenates. Moloney (13) applied a biochemical procedure to measurement of the LAP activity in rat leukocytes, and found a marked increase after whole-body irradiation. De Vries and Romijn (20) when making a cytochemical study of patients after X ray therapy and the staff of a hospital

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patient, or whether X-ray treatment had been given or not.

The arithmetical difference between the scores obtained after and prior to X-ray treatment was noted, and the mean differences were calculated for each group. The variability was compared with that in the control group, and the significance of the mean was evaluated by Student t-test.

Table II. Mean leukocyte alkaline phosphatase score in "normals" and in cancer patients

	No. of observ.	Mean score	S D	S. E. of mean
Normals	74	46.50	26.86	3.12
Cancer not treated	33	55.03	33.78	5.88
Cancer X-ray treated	25	43.52	36.86	7.37
All cancer patients	58	50.93	35.14	4.61

Results

Forty-five healthy people were tested, 29 of them twice. The mean LAP score of these 74 observations was 46.50. The normal range was very wide the lowest score was 4 and the highest 133 However the individual variance was much less. The standard deviation in 29 persons tested twice was 14.43 compared with 39.44 if no dependency was present. The correlation factor between two observations of one person was 0.86 It may be concluded that there is considerably less variability in one person tested at different times than between different persons.

N effect of irradiation was noted in 16 patients who received less than 3 000 The mean difference between the values obtained after and before treatment was very close to zero (— 0.38)

The average LAP score of cancer patients given X ray treatment 1—3 months before the observation was a little lower than in untreated cases. Nevertheless, the difference between the groups was not statistically significant and there was no significant difference noted between the cancer groups and the normal group (table II)

In both groups of cancer cases there occurred a slight increase of mean LAP value during X-ray treatment (table III) However the effect of irradiation varied substantially from case to case, as is illustrated by the high values for the standard deviation. In some cases, 2—3-fold increases of LAP were noted but in other cases there was no effect. In a few instances the LAP was lower after irradiation than before treatment. The average increase was not significant for respective groups, but almost significant for all the cancer patients taken together

The variability was more pronounced than the moderate average increase of LAP during X ray treatment. The average difference between two values in the irradiated cancer group was much larger

Table III LAP changes during X-ray treatment, statistical analysis

	No. of cases	Mean of diff.	S. E. mean diff	Risk	Mean abs.	S. D of diff.	Risk
Cancer not previously treated	33	+ 7.79	6.44	0.3	26.0	37.01	0.001
Cancer previously treated	25	— 10.16	5.10	0.1	21.4	25.48	0.01
All cancer patients	58	+ 8.81	4.15	0.05	24.0	31.8	0.001
Controls	29	— 1.00	2.68	0.6	11.2	14.43	—

Table I. The site of the tumor in cancer patients given X-ray treatment

Site of primary tumor	No. of pat.
Lung	20
Breast	16
Uterus	5
Esophagus	4
Ovary	4
Stomach	4
Thyroid gland	1
Bladder	1
Pharynx	1
Maxillary sinus	1
Reticulosarcoma	1

X ray department, noted decreased LAP activity after exposure.

The results of previous studies have thus been contradictory. However, as a simple cytochemical method would provide a valuable clinical tool if it could be used for the diagnosis of X ray damage, a study of the LAP in patients during X ray treatment seemed indicated.

Material and methods

The normals selected for this study comprised healthy blood donors and members of the hospital staff, in all 26 males and 19 females. Two blood samples were obtained at intervals of 3 days or more from 29 normals, and the normal variance was calculated. Seventy-four patients were tested before and after a series of X-ray treatments. The second sample was usually obtained either on the day of giving the last irradiation, or on the following day. In addition some patients were tested several times during the course of X ray treatment and after it, but the figures thus obtained are not included in the final material treated statistically. The irradiated patients are divided into three groups. Less than 3 000 r was given to those in the first group. The patients in the second group, who had not previously had X-ray treatment were given 3,000 r or more. Those in the third group were also subjected to 3 000 r or more, but they had already had one or several courses of X ray treatment, of which the most recent had usually been given 1—3 months previously. All the patients in the second and third groups were suffering from cancer. In most cases the cancer was inoperable, but in a few instances the tumor had been removed surgically. As is shown in table I, the most common diagnoses were pulmonary and mammary cancer. All cases with known infections were excluded.

The cancer patients were given conventional deep X-ray therapy using 180—200 kV and 1 mm Cu or Thoraeus filters. In most cases, 300—350 r was given every weekday during a period of two or three weeks. The number and the area of the fields varied according to the size and the location of the tumor. The average field was eight by ten cm, and the total skin dose during one course varied between 3 000 and 6,400 r.

Seven cancer patients were treated with cyclophosphamid, of which the average dose was 500 mg administered intravenously every second day, the total dose given during the observation period varying between 3 and 5 g.

About 10 ml of venous blood containing 50 units of heparin were centrifuged at 1 000 rpm for five minutes, and smears were prepared from the buffy coat. The dry smears were fixed in cold 10 % ethanol-formalin for one minute, and stained by Kaplow's (7) azo-coupling method as described by Merker and Heilmeyer (11). However, before the nuclear staining with Mayer's hematoxylin the smears were incubated for 6 min. at room temperature in a solution of 0.5 % celestine blue and 5 % ammonium ferric sulphate in water.

One hundred polymorphonuclear leukocytes were counted, and the "score" was calculated in the manner described by others (7, 11). However not more than three classes of positive cells were distinguished: 1 + light yellow brown granularity, 2 + more than half of the cell yellow brown, or a large number of brown granules, 3 + the whole cytoplasm dark brown and/or a part of the cytoplasm black. Leukocyte and differential counts were made from the same venous blood specimens. Almost all of the counts were carried out by the same person. The technician was not aware whether the sample had been obtained from a normal person or from a

patient, or whether X-ray treatment had been given or not.

The arithmetical difference between the scores obtained after and prior to X-ray treatment was noted, and the mean differences were calculated for each group. The variability was compared with that in the control group, and the significance of the mean was evaluated by Student t-test.

Results

Forty five healthy people were tested, 29 of them twice. The mean LAP score of these 74 observations was 46.30 The normal range was very wide the lowest score was 4 and the highest 133 However the individual variance was much less. The standard deviation in 29 persons tested twice was 14.43 compared with 39.44 if no dependency was present. The correlation factor between two observations of one person was 0.86. It may be concluded that there is considerably less variability in one person tested at different times than between different persons.

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The average LAP score of cancer patients given X-ray treatment 1—3 months before the observation was a little lower than in untreated cases. Nevertheless, the difference between the groups was not

Table II Mean leukocyte alkaline phosphatase score in "normals" and in cancer patients

	No. of observ	Mean score	S. D	S. E. of mean
Normals	74	46.30	26.86	3.12
Cancer not treated.	33	55.03	33.78	5.88
Cancer X-ray treated	25	45.52	36.86	7.37
All cancer patients	58	50.93	35.14	4 61

statistically significant, and there was no significant difference noted between the cancer groups and the normal group (table II)

In both groups of cancer cases there occurred a slight increase of mean LAP value during X-ray treatment (tabl III) However the effect of irradiation varied substantially from case to case, as is illustrated by the high values for the standard deviation. In some cases, 2—3-fold increases of LAP were noted but in other cases there was no effect. In a few instances the LAP was lower after irradiation than before treatment. The average increase was not significant for respective groups, but almost significant for all the cancer patients taken together

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Cancer previously treated	25	+ 10.16	5.10	0.1	21.4	25.48	0 01
All cancer patients	58	+ 8.81	4.15	0.05	24.0	31.58	0 001
Controls	29	— 1.00	2.68	0.8	11.2	14.43	—

than in the control series. The difference between the respective standard deviations is highly significant (table III) It thus seems evident that the LAP varies much more in irradiated cancer patients than in normals

In addition to the cases of cancer given X ray treatment, seven cancer patients were observed during the course of treatment with cyclophosphamid No consistent effect on the LAP values was noted and both increases and decreases were observable

Discussion

We have been unable to confirm the results reported by de Vries and Romijn (20) which indicate decreased LAP activity in people subjected to irradiation According to the findings of the present study there is usually an increase how ever this is slight, and is not observable in all cases. Consequently it cannot be accepted as a sign of X ray damage in a single case.

Our results agree with those obtained in a number of experimental studies (2, 10 13 17) but it should be noted that some experimental workers have achieved results which are quite the opposite (1 5) The disagreement in both experimental and clinical investigations might originate in a number of technical factors. In animal experiments usually the whole of the animal has been exposed but the dose administered has not been the same in all investigations, and the time of observation has differed In clinical studies the divergences are much more pronounced Observations of X ray staff cannot be compared with studies of cancer patients undergoing X ray treatment The subjects differ too widely as do the daily doses and the periods of exposure. The discrepancy between the results obtained by de Vries and Romijn with irradiated cancer patients and those achieved by us, may find their explanation in several X ray technical factors. These authors do not mention the daily doses, the total time of treatment nor other technical data. The field usually varies from case to case, and so does the dose exerting a direct effect upon the hematopoietic organs. The last mentioned factor is probably of great importance, and might to some extent explain the varying results in different cases. The time factor is also important In the present investigation, a few of the cases were followed for a lengthy period during and after the X-ray treatment, but as no consistent time relationship was noted we decided to obtain the post irradiation specimen immediately subsequent to the last exposure. However we do not know whether the value thus obtained is the most representative one.

The LAP activity varies from cell to cell It is thought to increase with increasing maturity (18, 21) If irradiation inhibits the production of young granulocytes, the average age of the granulocytic population is assumed to increase after exposure. A general increase of LAP activity per cell could thus be easily explained but no relationship was observed between LAP and the leukocyte count or the differential court.

The most significant difference between the cancer group and the control group is not the moderate average increase of LAP in the irradiated cases, but the variability If we compare two values in the treated cancer patients, obtained before and after treatment respectively with two values in the controls, we find an appreciably greater variability in the former group There thus seems to exist a

marked individually different radiation response as regards LAP some people react with increased enzyme activity others with decreased values, and those of a third group show no response. In view of the number of factors involved, however this conclusion might well be incorrect. It is probable that the most important of these factors are the direct irradiation of red bone marrow in different cases, and the progress of the disease during the observation period. In all probability a study of the individual response, with these factors taken into consideration, would be worthwhile. The availability of a large series of patients is essential and a control series of untreated cancer patients must be included.

Summary

The leukocyte alkaline phosphatase (LAP) of venous blood has been evaluated by cytochemical means, and the "score" calculated. The mean value of 74 observations in "normals" was 46.3 ± 26.9 The average score in 58 cancer patients did not differ significantly from the normal value. A moderate increase was usually noted immediately after a course of X-ray treatment of 3,000 r or more, but many exceptions were noted. The individual variability was much more pronounced in irradiated cancer patients than in untreated healthy people. An X-ray dose of less than 3,000 r had no effect on LAP in 16 patients nor did treatment with cyclophosphamid in the seven cases observed.

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A New Quinidine Preparation with Sustained Release

By

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Although newer compounds have been suggested for the treatment of atrial fibrillation, quinidine is still the drug of choice. The need of multiple and rather high doses to achieve effect (5, 18) has led to the development of long-acting quinidine preparations (1, 8, 10, 11) such that fewer doses are needed daily. A recent report to the Council on Drugs of the American Medical Association stated, however, that such extended-action preparations are too irregular in absorption to be relied upon in critical situations (9).

The present study describes a new type of sustained release quinidine tablet, Durettes (12, 15, 16, 17), where quinidine bisulfate has been embedded in a porous, insoluble plastic tablet. The tablet used released about 40 % of the dose in the first hour and the rest steadily for about 8 hours tested in vitro according to Sjögren (14). Quinidine is probably not absorbed in the stomach (6) but rapidly absorbed in the alkaline milieu of the small intestine (13). Excellent absorption even more distally in the intestine was demonstrated by Sampson et al. (11) in a patient with colostomy in the descending colon. It is also partly absorbed when administered by rectum (7). Quinidine might, therefore, be suitable for a sustained release preparation. Quinidine bisulfate is more soluble in water than quinidine sulfate and therefore preferable pharmaceutically.

Material and methods

Concentration of quinidine in plasma after single doses

Ten hospitalized patients were studied, 8 with atrial fibrillation and 2 with premature beats. Their body weight varied from 57 to 89 kg. None of the patients was in congestive heart failure. All had normal renal function except one with hypertension and serum creatinine of 1.8 mg% (patient M 51, fig. 1 top, left).

With 2—4 days interval equivalent single doses of sustained release tablets (0.125 g quinidine bisulfate/10 kg body weight) and ordinary tablets (0.1 g quinidine sulfate/10 kg body weight) were administered together with 20—40 ml water to the fasting patient in the morning. Blood samples were drawn before the dose and then every 15 min. during the first 3 hours, every 30 min. during the next 2 hours and then 6, 8, 10, 12 and 24

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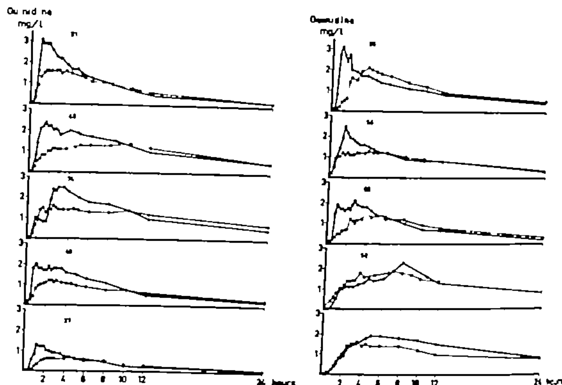


Fig 1 Concentration of quinidine in plasma in 10 patients after equivalent single doses of
○ = ordinary tablets (0.1 g quinidine sulfate/10 kg body weight).
● = sustained release tablets (0 125 g quinidine bisulfate/10 kg body weight)

hours after the dose. The patients received a standard meal, 2 buttered biscuits and a can of tomato- or mushroom soup, 3 hours after the quinidine dose, and the ordinary hospital dinner 8 hours after the dose.

Two of the patients some days later received a single but doubled dose of Duretter (0.25 g quinidine bisulfate/10 kg body weight)

The venous blood samples were drawn from an indwelling needle. The quinidine in plasma was analysed according to a double-extraction method of Brodie and Udenfriend (2 3) elaborated by Cramér and Isaksson (4)

Morning concentration of quinidine in plasma during long-term treatment

Seven out patients with restored sinus rhythm after atrial fibrillation received quinidine in ordinary tablets for at least 3 months and thereafter in sustained release form. The daily doses were adjusted to the need of the individual patient and varied from 1.2 to 2 0 g quinidine sulfate. The ordinary tablets were administered in 4 equal doses daily 8, 12 a. m. and 4 8 p. m. The same daily amount of quinidine was given to the individual patient as sustained release tablets twice daily in equal doses, 8 a. m. and 8 p. m.

The concentrations of quinidine in plasma were determined on three consecutive mornings at the end of the first three months' period. Then the therapy was changed and after 2 weeks of sustained release medication the concentrations of quinidine were again determined on three consecutive mornings. After two months blood samples were drawn again on two consecutive days. The samples were always taken at 8 a. m. before the morning dose.

Conversion of atrial fibrillation

Sustained release quinidine tablets were tried for conversion of atrial fibrillation to sinus rhythm in 18 patients. Three patients had essential atrial fibrillation, 8 had atherosclerotic heart disease and/or hypertensive heart disease and 7 had rheumatic heart disease in connection with atrial fibrillation. All patients were pretreated with dicumarol and

Table I. Maximum concentration of quinidine in plasma in 10 patients after equivalent single doses of sustained release tablets and ordinary tablets (0.125 g quinidine bisulfate and 0.1 g quinidine sulfate/10 kg body weight, respectively)

Sex	Age (yrs)	Body weight (kg)	Max. conc. of quinidine in plasma			Max. conc. reached	
			SRT	OT	SRT in % of OT	SRT	OT
			mg/l			Hours after administration	
♂	51	64	1.6	3.1	51.6	1.50	1.00
♂	46	62	1.4	2.4	58.3	10.00	1.50
♂	74	88	1.6	2.5	64.0	2.50	3.00
♂	40	57	1.2	2.0	60.0	2.25	1.00
♂	57	61	0.7	1.3	53.8	4.50	1.25
♂	66	75	2.1	3.1	67.7	3.75	1.00
♂	56	70	1.3	2.5	52.0	4.00	1.50
♂	62	70	1.5	2.1	71.4	4.00	2.75
♀	52	71	1.9	2.3	82.6	7.00	8.00
♂	51	87	1.6	1.9	84.2	3.50	5.00
		Mean values	1.5	2.3	64.6	4.30	2.60
		Median values	—	—	—	3.88	1.50

SRT = sustained release tablets OT = ordinary tablets.

stayed in bed during the quinidine-medication. A 2 doses-a-day schedule was used, each dose of sustained release tablets generally being 1.5 g quinidine bisulfate. The tablets were given at 6 a. m. and 2 p. m. Every day the plasma level of quinidine was determined at 6 a. m. before the morning dose and 3—4 hours after the dose at 2 p. m. The electrocardiogram was recorded at least twice daily before the quinidine doses, and in addition when conversion to regular rhythm was noticed.

Long-term treatment after conversion

Maintenance therapy with sustained release quinidine tablets was studied in 38 patients with sinus rhythm after conversion of atrial fibrillation. Thirteen of the patients had been converted by sustained release tablets and 25 by ordinary quinidine sulfate tablets. Sixteen patients had essential fibrillation or arteriosclerotic heart disease and 9 had rheumatic heart disease.

During long-term treatment the sustained release tablets were given in equal doses daily with 12-hour intervals. The total daily dose varied from 1.0 to 3.0 g quinidine bisulfate. The observation period is at present 2 to 8 months.

Results

Concentration of quinidine in plasma after single doses

After single doses there were varying individual responses in plasma quinidine concentration, therefore all 10 patients are presented in fig. 1. A 24-hour specimen could not be obtained from patient F 52. Maximum concentrations and the time between administration and maximum concentration are given in table I.

In a given patient the concentration of quinidine in plasma differed after equivalent doses of the two preparations. After ordinary tablets there was in 8 patients out of 10 an early high maximum concentration and in every patient, directly after the maximum concentration a descending slope of the concentration curve. After sustained release tablets there is no high maximum concentration. After an ascending limb of the curve, the quinidine concentration in 7 patients

Table II Duration in minutes of 90 80 70 and 60 % of the maximum concentration of quinidine in plasma after equivalent single doses of sustained release tablets and of ordinary tablets (0 125 g quinidine bisulfate and 0 1 g quinidine sulfate/10 kg body weight respectively)

Patient		90 %			80 %			70 %			60 %		
Sex	Age (yrs)	SRT	OT	Diff SRT OT	SRT	OT	Diff SRT-OT	SRT	OT	Diff. SRT-OT	SRT	OT	Diff. SRT OT
		Minutes											
♂	51	186	58	+128	262	81	+181	306	134	+172	454	174	+280
♂	46	403	53	+350	573	113	+460	825	326	+499	1,005	495	+510
♂	74	48	102	− 54	604	162	+442	792	277	+515	1,002	432	+570
♂	40	114	102	+ 12	210	198	+ 12	342	291	+ 51	[illegible]	384	[illegible]
♂	37	36	50	− 14	198	60	+138	384	98	+286	433	167	+266
♂	66	97	24	+ 73	208	56	+152	342	74	+268	460	117	+343
♂	56	240	21	+219	396	38	+358	520	86	+434	703	117	+588
♂	62	33	38	− 5	276	170	+106	396	243	+153	474	333	+141
♀	52	210	81	+129	402	172	+230	600	261	+339	—	—	—
♂	51	105	324	−219	402	492	− 90	504	744	−240	708	960	[illegible]
Mean of diff.		+ 62			+ 199			+ 248			+ 294		
S.D of diff.		157			179			229			260		
t		1.24			3.52			3.42			3.39		
df		9			9			9			8		
P		20% < P < 30%			0.1 % < P < 1 %			0.1% < P < 1 %			0.1% < P < 1 %		
Confidence interval (95 %)		62 ± 112			199 ± 128			248 ± 164			294 ± 200		

SRT = sustained release tablets. OT = ordinary tablets. Diff = Difference.

makes a horisontal plateau before it assumes a descending slope.

The concentrations of quinidine in plasma after sustained release tablets show no more fluctuations than after ordinary tablets

The maximum concentration of quinidine in plasma was in every patient lower after sustained release tablets, with a mean value of 1 5 mg/l compared to 2.3 mg/l after ordinary tablets (table I) The maximum concentration with sustained release tablets in per cent of the maximum concentration with ordinary tablets is given in table I The mean value of these percentages is 64 6 % Thus mean is statistically different from 100 (t = 9 43, df = 9 P < 0 1 %) The confidence interval (95 %) for the mean is 64.6 ± 8.5 % The median time to reach maximum concentration was 3 88 hours after sustained release tablets and 1.50 hours after ordinary tablets. The median values are more appropriate than the mean values because of the skew distribution (table I)

The time during which the plasma concentration exceeded 90 80 70 and 60 % of the maximum concentration of quinidine in plasma was calculated for ordinary tablets and for sustained release tablets from fig 1 Sustained release tablets maintained 80 70 and 60 % of the

Table III. Statistical evaluation of table II with the two last patients excluded

	90 % Diff. SRT-OT	80 Diff. SRT-OT	70 % Diff. SRT-OT	60 % Diff. SRT-OT
Mean of diff.	+ 89	+ 231	+ 297	+ 363
S. D. of diff	138	167	171	172
	1.82	3.92	4.91	5.96
df	7	7	7	7
P	10% < P < 20%	0.1% < P < 1%	0.1% < P < 1%	P < 0.1%
Confidence interval (95%)	89 ± 115	231 ± 139	297 ± 143	363 ± 144

SRT = sustained release tablets. OT = ordinary tablets. Diff. = difference.

Table II. Concentration of quinidine in plasma at 8 a.m. with ordinary tablets, 4 doses daily and sustained release tablets, 2 doses daily. The total daily doses of ordinary tablets and of sustained release tablets were equivalent in the same patient

Sex	Age (yrs)	Body weight (kg)	Dose (g) OT 4 doses daily	Dose (g) SRT 2 doses daily	8 a.m. conc. of quinidine in plasma (mg/l) OT after 3 months treatment 3 consec. morn.		SRT after 1 week's treatment 3 consec. morn.		SRT after 2 months' treatment 2 consec. morn.	
♂	68	81	0.5	1.25	2.5 2.5 1.9	mean 2.3	3.5 3.1 3.2	mean 3.3	3.0 2.6	mean 2.8
♂	58	81	0.4	1.00	2.7 2.9 2.7	2.8	2.7 2.5 2.4	2.5	2.5 3.5	3.0
♀	42	79	0.4	1.00	3.8 3.8 3.2	3.6	2.9 2.5 2.7	2.7	3.0 3.4	3.2
♂	60	73	0.4	1.00	1.8 1.8 1.9	1.8	2.0 2.1 2.3	2.1	2.4 2.2	2.3
♂	61	68	0.4	1.00	2.5 2.1 2.2	2.3	2.1 1.7 2.0	1.9	2.4 2.6	2.5
♂	52	61	0.4	1.00	1.6 1.6 1.6	1.6	1.9 1.4 1.5	1.6	1.8 2.0	1.9
	62	49	0.3	0.75	2.2 2.1 2.3	2.2	2.0 1.7 2.1	1.9	1.2 1.1	1.2
				Mean values		2.4		2.3		2.4
				Range		1.6–3.6		1.6–3.3		1.2–3..

OT = ordinary tablets; SRT = sustained release tablets; consec. morn. = consecutive mornings.

Table II Duration in minutes of 90 80 70 and 60 % of the maximum concentration of quinidine in plasma after equivalent single doses of sustained release tablets and of ordinary tablets (0.125 g quinidine bisulfate and 0 1 g quinidine sulfate/10 kg body weight respectively)

Patient		90 %			80 %			70 %			60 %		
Sex	Age (yrs)	SRT	OT	Diff SRT-OT	SRT	OT	Diff SRT-OT	SRT	OT	Diff SRT-OT	SRT	OT	Diff SRT-OT
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♂	74	48	102	− 54	604	162	+442	792	277	+515	1,002	432	+570
♂	40	114	102	+ 12	210	198	+ 12	342	291	+ 51	586	334	+252
♂	37	36	50	− 14	198	60	+138	384	98	+286	433	167	+266
♂	66	97	24	+ 73	208	56	+152	342	74	+268	460	117	+343
♂	56	240	21	+219	396	38	+358	520	86	+434	705	117	+588
♂	62	33	38	− 5	276	170	+106	396	243	+153	474	333	+141
♀	52	210	81	+129	402	172	+230	600	261	+339	—	—	—
♂	51	105	324	−219	402	492	− 90	504	744	−240	708	960	−25[illegible]
Mean of diff.		+ 62			+ 199			+ 248			+ 294		
S.D of diff.		157			179			229			260		
t		1.24			3.52			3.42			3.39		
df		9			9			9			8		
P		20% < P < 30%			0 1% < P < 1 %			0.1% < P < 1%			0.1 < P < 1		
Confidence interval (95%)		62 ± 112			199 ± 128			248 ± 164			294 ± 900		

SRT = sustained release tablets. OT = ordinary tablets. Diff = Difference.

makes a horisontal plateau before it assumes a descending slope

The concentrations of quinidine in plasma after sustained release tablets show no more fluctuations than after ordinary tablets.

The maximum concentration of quinidine in plasma was in every patient lower after sustained release tablets, with a mean value of 1 5 mg/l compared to 2.3 mg/l after ordinary tablets (table I) The maximum concentration with sustained release tablets in per cent of the maximum concentration with ordinary tablets is given in table I The mean value of these percentages is 64 6 % This mean is statistically different from 100 (t = 9 43, df = 9 P < 0 1 %) The confidence interval (95 %) for the mean is 64 6 ± 8.5 %. The median time to reach maximum concentration was 3 88 hours after sustained release tablets and 1.50 hours after ordinary tablets. The median values are more appropriate than the mean values because of the skew distribution (table I).

The time during which the plasma concentration exceeded 90 80, 70 and 60 % of the maximum concentration of quinidine in plasma was calculated for ordinary tablets and for sustained release tablets from fig 1 Sustained release tablets maintained 80 70 and 60 % of the

Table III Statistical evaluation of table II with the two last patients excluded

	90 % Diff. SRT-OT	80 % Diff. SRT-OT	70 % Diff. SRT-OT	60 % Diff. SRT-OT
Mean of diff.	+ 89	+ 231	+ 297	+ 363
S. D. of diff.	138	167	171	172
t	1.82	3.92	4.91	5.98
df	7	7	7	7
P	10% < P < 20%	0.1% < P < 1%	0.1% < P < 1%	P < 0.1%
Confidence interval (95%)	89 ± 115	231 ± 139	297 ± 143	363 ± 144

SRT = sustained release tablets. OT = ordinary tablets. Diff. = difference.

Table IV Concentration of quinidine in plasma at 8 a.m. with ordinary tablets, 4 doses daily and sustained release tablets, 2 doses daily. The total daily doses of ordinary tablets and of sustained release tablets were equivalent in the same patient

Sex	Age (yrs)	Body weight (kg)	Dose (g) OT 4 doses daily	Dose (g) SRT 2 doses daily	8 a.m. conc. of quinidine in plasma (mg/l) OT after 3 months' treatment 3 consec. morn.		SRT after 1 week treatment 3 consec. morn.		SRT after 2 months treatment 2 consec. morn.	
♂	68	81	0.5	1.25	2.5	mean 2.3	3.5	mean 3.3	3.0	mean 2.8
					2.5		3.1		2.6	
					1.9		3.2			
♂	58	81	0.4	1.00	2.7	2.8	2.7	2.5	2.5	3.0
					2.9		2.5		3.5	
					2.7		2.4			
♀	42	79	0.4	1.00	3.8	3.6	2.9	2.7	3.0	3.2
					3.8		2.5		3.4	
					3.2		2.7			
♂	68	75	0.4	1.00	1.8	1.8	2.0	2.1	2.4	2.3
					1.6		2.1		2.2	
					1.9		2.3			
♂	61	68	0.4	1.00	2.5	2.3	2.1	1.9	2.4	2.3
					2.1		1.7		2.5	
					2.2		2.0			
♂	32	61	0.4	1.00	1.6	1.6	1.9	1.6	1.8	1.9
					1.6		1.4		2.0	
					1.6		1.5			
♀	62	49	0.3	0.75	2.2	2.2	2.0	1.9	1.2	1.2
					2.1		1.7		1.1	
					2.3		2.1			
				Mean values		2.4		2.3		2.4
				Range		1.6—3.6		1.4—3.3		1.2—3.2

OT = ordinary tablets SRT = sustained release tablets consec. morn. = consecutive mornings.

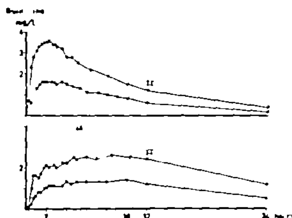


Fig 2. Concentration of quinidine in plasma after single doses of sustained release tablets.

I ● – 0.125 g quinidine bisulfate/10 kg body weight.

II ● – 0.25 g quinidine bisulfate/10 kg body weight.

maximum concentration on the average for 199 248 and 294 minutes longer respectively than ordinary tablets (table II) The differences were significantly different from 0 (P < 1 %) At the 90 % level of the maximum concentration significant differences were not attained because of a wide dispersion in the material

The last two patients in fig 1 differed from the others by a slow absorption of quinidine even after ordinary tablets. A statistical calculation of table II with these two patients excluded is shown in table III Sustained release tablets maintained 80 70 and 60 % of the maximum concentration of quinidine in plasma on the average 231 297 and 363 minutes longer than ordinary tablets. The differences are significant (P < 1 % P < 1 % and P < 0 1 %) respectively)

The 2 patients who also received a single but doubled dose of sustained release tablets are shown in fig 2 In the individual patient the concentration curve of quinidine in plasma showed almost the same pattern after 0 125 and 0.25 g quinidine bisulfate/10 kg body weight, but the concentration of quinidine was about twice as high after the double dose for 1 to 24 hours after the dose.

Comparison of the maximum concentrations in fig 2 with the maximum concentrations for the same patients (M 51 and M 46) in fig 1 discloses that the doubled dose of sustained release tablets did not cause an appreciably higher level than the peak concentration after the single dose of ordinary tablets.

Morning concentration of quinidine in plasma during long-term treatment

During maintenance therapy in 7 out patients the concentrations of quinidine in plasma at 8 a. m before the morning dose were the same with sustained release tablets twice daily as with ordinary tablets of quinidine sulfate 4 times daily when the total daily dose of quinidine was equivalent (table IV)

Conversion of atrial fibrillation

In 13 of 18 patients with atrial fibrillation, normal sinus rhythm was achieved by sustained release tablets with a 2 doses-a-day schedule in 1 to 4 days with a maximum concentration of quinidine in plasma varying from 2.5 to 7 0 mg/l (table V) No side-effects of quinidine were noticed except slight gastrointestinal symptoms in 3 patients.

Long-term treatment after conversion

Eleven patients of 13 converted to sinus rhythm by sustained release tablets, retained normal sinus rhythm with sustained release tablets in 2 doses daily during an observation period of more than 1 month Two of the patients relapsed to atrial fibrillation in 1 to 3 weeks, one because of nausea and diarrhea and

Table V. Conversion to sinus rhythm, maximum concentration of quinidine in plasma before conversion and applied doses of sustained release tablets in 18 patients with atrial fibrillation

Sex	Age (yrs)	Origin of atrial fibrillation	Max. conc. of quinidine in plasma before conversion (mg/l)	Conversion to sinus rhythm	SRT	
					Daily (g)	Days
♀	57	Unknown	—	+	1.0 + 1.0	1
♂	64	Unknown	—	+	1.5	0.5
♂	56	Unknown	3.0	+	1.5 + 1.5	2
♂	67	AHD	7.0	—	1.5 + 1.5	2
					2.0 + 2.0	1
♂	66	AHD	2.8	(+)	0.75 + 0.75	1
♂	45	AHD	5.5	+	1.0 + 1.0	1
♀	54	AHD + TT	3.6	+	1.5	0.5
♀	59	HHD	4.7	—	1.5 + 1.5	1.5
♀	68	HHD	2.5	+	1.5 + 1.5	1
♀	58	HHD	4.1	+	1.5 + 1.5	2
♀	63	HHD	—	+	0.5 + 0.5	3
					1.0 + 1.0	1
♂	51	MS	5.5	—	1.5 + 1.5	5
♂	48	MS op.	5.2	+	1.5 + 1.5	1
					1.5 + 0.75	1
					1.0	0.5
♂	46	MS op.	4.8	(+)	1.5 + 1.5	2.5
♂	42	MS op.	3.6	+	1.5	0.5
♀	46	MS op.	6.0	+	1.5 + 1.5	2
					1.5 + 1.0	1.5
♂	41	MS op. + MI	3.4	+	1.5 + 1.5	1
♀	31	MS op. + AS	3.0	+	1.5 + 1.5	1

AHD = atherosclerotic heart disease TT = thyrotoxicosis HHD = hypertensive heart disease
MS = mitral stenosis MI = mitral insufficiency AS = aortic stenosis op. = operated
( ) denotes transition to atrial flutter within 24 hours; SRT = sustained release tablets.

one probably due to low maintenance therapy (0.75 g quinidine bisulfate, 2 doses daily). After 2 months of normal sinus rhythm one patient developed urticaria, fever and granulocytopenia. Quinidine medication was therefore discontinued and atrial fibrillation returned 2 or 3 weeks later. In another patient the quinidine had to be withdrawn after 6 months of normal sinus rhythm because of thrombocytopenia. Atrial fibrillation returned 2 weeks later.

In the other 25 patients, where the maintenance therapy was changed from ordinary tablets, 4 doses daily, to sustained release tablets, 2 doses daily, no one developed complications and all retained normal sinus rhythm during an observation period of 2 to 8 months. All the patients found the 2 doses-a-day schedule more convenient to adhere to. At present 7 patients have less gastrointestinal side-effects with sustained release tablets than with ordinary tablets.

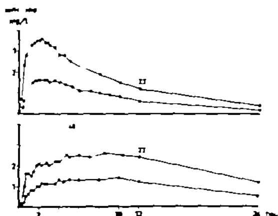


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Discussion

The present study demonstrates that the quinidine concentration in plasma after sustained release tablets differs significantly from that after an equivalent dose of quinidine in ordinary tablets.

The maximum concentration is significantly lower and the peak concentration seen after ordinary tablets is replaced by a plateau which develops gradually and eventually falls off. The ascending part and the horizontal plateau part of the curve suggest that the release of quinidine is slow and prolonged from these sustained release tablets in man. The absence of fluctuations of concentration on the ascending and horizontal parts of the curve indicates that the release is regular with respect to time and reliable with respect to different subjects.

The descending part of the curve represents mainly the elimination of the quinidine from the plasma and has the same slope as in curves after ordinary tablets. The elimination of quinidine is thus not affected by sustained release tablets.

The same maximum concentration of quinidine can be obtained by sustained release tablets as by ordinary tablets with an approximately double dose of the former. The maximum concentration is attained more slowly and remains virtually unchanged during a significantly longer period of time than after ordinary tablets. Thus a therapeutically effective plasma concentration can be kept constant and maintained during a longer time.

During long term treatment in comparison with ordinary tablets sustained release tablets gave the same morning concentration of quinidine in plasma with fewer daily doses, which makes them more convenient for the patient to use. Repeated high peaks and marked fluctuations in plasma concentration are avoided with sustained release tablets. It can be assumed that this is of importance with regard to side effects. Our present experience supports this assumption.

In conversion of atrial fibrillation to normal sinus rhythm a dependable clinical result was obtained without complications by sustained release tablets, 2 doses daily.

As maintenance therapy after conversion, sustained release tablets have an adequate antiarrhytmic effect with more convenient administration by 2 doses daily, morning and evening.

Summary

1. The concentration of quinidine in plasma after sustained release tablets differs from that after an equivalent dose of ordinary tablets. The maximum concentration after sustained release tablets is significantly lower and remains unchanged during a significantly longer period. The horizontal plateau part of the concentration curve suggests a sustained release of quinidine.

2. The morning concentration of quinidine in plasma during long-term treatment was the same with sustained release tablets in 2 doses daily as with ordinary tablets in 4 doses daily, when the total daily dose of quinidine was equivalent.

3. Thirteen of 18 patients with atrial fibrillation were converted to normal sinus rhythm by sustained release tablets without complications.

4. Eleven of these 13 patients maintained normal sinus rhythm by sustained release tablets, 2 doses daily, during an observation period of more than 1 month. In 25 patients the maintenance therapy was changed from ordinary tablets, 4 doses daily, to sustained release tablets, 2 doses daily. No relapse to atrial fibrillation was observed after 2 to 8 months.

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An Attempt to Localize Macroglobulins by Means of Paper Electrophoresis

By

Børge Larsen and Jørgen Lyngbye

During a study of experimental amyloidosis in splenectomized rabbits, electrophoretic blood serum analyses showed an abnormal distribution of γ-globulin after periodic-acid Schiff staining (P.A.S) Ultracentrifuge analyses of these sera revealed an increase in the S 19 macroglobulin content that was suggested to be rel ted to the electrophoretic finding

The macroglobulins in serum have been shown to contain considerable amounts of protein-bound carbohydrates. According to Heremans (4) the macroglobulins contain about 10 of this component. Electrophoretically the macroglobulins discussed here belong to the fast migrating γ-globulins. These data would suggest that a high macroglobulin content may contribute to an elevation of protein-bound carbohydrate in the position between γ- and β-globulin. However such variations in electrophoretic mobility of protein-bound carbohydrate due to the presence of macroglobulin might be too minute to be demonstrated by means of customary paper electrophoresis, where two parallel runs are compared after one of the strips has been stained for protein and the other for glycoprotein.

It was therefore considered of interest to determine if any displacement of the glycoprotein distribution in the β- γ-globulin area could be demonstrated by applying a staining method for paper electrophoresis, where the same strip is stained for both protein and protein-bound carbohydrate. For this purpose sera from five patients with macroglobulinemia were studied. Furthermore the investigation included sera from patients with rheumatoid arthritis demonstrating a positive latex fixation test as a sign of presence of rheumatic factor

The method which can be utilised for this purpose is described, and it is fou d that sera from rabbits with experimental inflammation exhibit a displacement in the glycoprotein pattern, which is compatible with the assumption that the

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macroglobulins are located between the β- and γ-globulins. However no such displacement could be demonstrated in sera from the patients studied even though macroglobulins have been demonstrated in sera from five of the patients.

Material

ANIMALS

White female New Zealand rabbits weighing between 3.0 and 3.5 kg were employed for the study. The animals were divided into three groups. A. Treated intact animals. B. Splenectomized animals. C. Normal animals (controls).

Group A (2 animals)

Each rabbit in this group received 5 ml of an aqueous suspension of sodium caseinate[1] (10 %) subcutaneously at regular intervals twice a week. The suspension was freshly prepared before each injection and no antibiotic agent or preservative was added. Blood samples (venous blood) were taken without addition of anticoagulants from the ear every second day. Blood serum was stored at —20° C.

Group B (2 animals)

Splenectomy was performed under Nembutal anesthesia, each animal receiving intravenously 36 mg Sodium Pentbarbitol® per kg of body weight. The animals were permitted to rest for one month after the operation before the start of the experiment. From that time the animals were treated exactly as described for group A.

Group C (17 animals)

Normal (control) rabbits were bled according to the same schedule as described for group A and B. Pooled normal serum was used for the study.

[1] Sodium caseinate Lot. No. 421130 obtained from the Matheson Company Inc., Norwood, Ohio, U.S.A.

All animals in the three groups were maintained on a diet of standard Purina Rabbit Chow (containing 15 % protein and necessary minerals and vitamins) and water ad libitum.

PATIENTS

The patient material was divided into three groups according to the diagnosis: 1. Primary macroglobulinemia (Waldenström). 2. Secondary macroglobulinemia. 3. Rheumatoid arthritis.

Group 1 (3 patients)

All three patients suffered from verified macroglobulinemia Waldenström. Blood serum was kindly supplied by Professor Knud Brochner Mortensen and Dr Aage Drivsholm, Medical Department A, Rigshospitalet, Copenhagen.

Group 2 (2 patients)

Both patients had a secondary macroglobulinemia, which immunoelectrophoretically was of the β_{2M}-type. One of the patients (case 4) also had a severe diabetes mellitus of about 30 years duration and a diabetic nephropathia. The other patient (case 5) suffered from a severe nephrotic syndrome and died after six months stay in the ward. These patients were from the First and Second Medical Department, Århus Kommunehospital, Århus.

Group 3 (14 patients)

These suffered from rheumatoid arthritis and only patients with a positive sensitized sheep-cell agglutination and/or latex fixation test were used for the study. Blood serum and clinical information from twelve of the patients were obtained by courtesy of Dr Holger Jacobsen, Danish Red Cross "Folkekurved Hald" while two patients were from the First Medical Department, Århus Kommunehospital.

One to three blood samples (venous blood) were taken without addition of anticoagulants from all patients in the three groups during their stay in hospital, and blood serum was stored at —20 C.

Blood serum from twenty normal persons served as control material. These were kindly supplied by Dr F. Kissmeyer Nielsen, head of the Blood Bank and Blood Grouping Laboratory, Århus Kommunehospital.

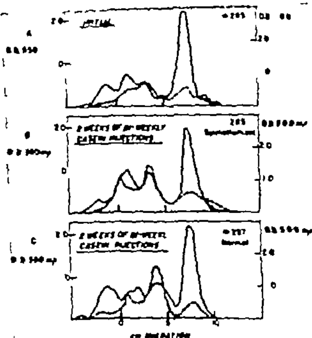


Fig. 1. Paper electrophoretic serum-protein pattern and distribution of P.A.S. stainable material in rabbit serum. Photometric densitograms of amido black 10 B measured at 500 mμ and fuchsin sulfite measured 550 mμ from successive stainings with amido black 10 B and P.A.S. on paper electrophoretic strips. A. Splenectomized rabbit before casein injections. B. Splenectomized rabbit after 2 weeks of casein treatment. C. Non-splenectomized rabbit after 2 weeks of casein injections. Note the prominent P.A.S. peak in the β-globulin fractions from the treated rabbits.

Methods

Paper electrophoresis was performed on Schleicher & Schull paper 2043 B with Tris, EDTA, borate buffer pH 8.9. The potential gradient was 5.0 V/cm during the 20-hour run at room temperature (1). Serum proteins were stained with amido black 10 B and the electrograms were copied on Agfa orthochromatic negative film. The amido black 10 B was removed by repeated washings in a solution of 2.5 % sulfosalicylic acid in 96 % ethanol. Five washings each of 30 minutes duration were usually sufficient to remove the last traces of amido black. After the last washing the paper strips were rinsed twice with 96 % ethanol. The next step was staining for protein-bound carbohydrate with a modified P.A.S. reaction with "neufuchsin" (6, 11). As soon as possible after the P.A.S. staining the strips were copied again on Agfa film. The film copies from both staining procedures were scanned in an Elphor H photoelectric colorimeter.

Ultracentrifuge diagrams for the patients' sera were obtained by courtesy of civil engineer Robert Djurtoft, Carlsberg Bryggeriernes Laboratorium, Copenhagen and immunoelectrophoresis was done by Dr. Jørgen Clausen, Biochemical Institute, University of Copenhagen (2).

Results and discussion

A. The method for double staining on paper electrophoresis strips

Although the maximal absorbance with amido black and that with fuchsin sulfite resulting from the P.A.S. staining procedure differ by 50 mμ (500 mμ for amido black and 550 mμ for fuchsin sulfite) it is not possible to determine both colors on one strip stained with these two stains. The procedure which we have used — initial application of the protein stain and then a rinse in sulphosalicylic acid dissolved in ethanol followed by P.A.S. staining — has been tested with respect to possible loss of protein during the staining procedures.

Table I Ultracentrifuge and immuno-electrophoretic analyses in patients with primary and secondary macroglobulinemia (group 1 and 2) Cases no I—III were suffering from primary and cases no IV and V from secondary macroglobulinemia

Case	Age	Sex	Sedimentation constant f macroglobulins	% of each macroglob. fraction	Immunoelectrophoresis
I	69	♂	17.3 S 22.7 S	15.7 5.2	β_{2M} paraprotein
II	70	♂	13.6 S 20.3 S	16.7 7.8	β_{2M}-paraprotein
III	44	♂	12.2 S 16.0 S 17.5 S 19.3 S	7.6 1.9 1.4 2.9	β_{2M}-paraprotein
IV	57	♀	14.6 S 25.6 S	9.6 5.8	Increase of β_{2M}
V	60	♀	13.6 S	12.4	Increase of β_{2M}

Of the total serum protein content.

The rinsing in sulfosalicylic acid does not result in any loss of protein from the strips as protein-stained strips after this treatment could be restained with amido black and thereby exhibit a protein pattern identical to that obtained before rinsing Similarly the protein staining procedure with subsequent rinsing does not alter the intensity of the P.A.S stain when this is compared to the intensity of the same stain applied without previous protein staining

The reverse sequence of staining operations, however cannot be used Strips stained with P.A.S are easily bleached in 0 5 % KOH in 99 % ethanol It is therefore easy to eliminate the P.A.S stain in strips after they have been stained for protein It was noted that if the P.A.S staining is done before the protein staining a loss of 40—50 % of the initial protein-staining intensity of the serum albumin is seen. This loss was not due to the exposure to the alkaline ethanol (10)

B Application of the double staining method to blood serum from rabbits and patients

Ultracentrifuge analysis of pooled serum from normal rabbits showed a macroglobulin (S 19) content of 2.6 % of the total serum protein After splenectomy and casein injections the S 19 macroglobulin component increased to 4.9 %. A less pronounced increase was seen in the non-splenectomized casein-treated animals. Fig I shows the results of electrophoresis of serum from an untreated animal and from the same rabbit after splenectomy and two weeks of casein treatment. It is noted that the P.A.S. positive glycoprotein pattern depicted by the gray area in the figure exhibits a displacement in the β- γ-globulin area. A new P.A.S stained peak appeared between β and γ-globulin. This was also the case for a non-splenectomized casein-treated rabbit as seen on diagram C in fig 1 The intensity of this peak seems to be in accordance with the content of macroglobulin judged from ultracentrifugation studies.

In humans the normal macroglobulin component of blood serum rarely exceeds 3—5 % of the total protein content (5) and it is mainly of the α-globulin type contrary to the pathological macroglobulins which often are of a fast moving γ-globulin type (7) Consequently it was tempting on the basis of the results from the experiments on rabbits to investigate human sera with proved macroglobulinemia. Table I shows the results of ultracentrifuge and immuno-electrophoretic analysis on sera from patients with

Fig. 2. Paper electrophoresis pattern of serum from patient no. III with primary macroglobulinemia Waldenström stained with amido black 10 B (top) and P.A.S. (bottom). The γ-globulin is on the left side.

Fig. 3. Serum electrophoresis pattern of a normal person (N) and of patient (case III) with primary macroglobulinemia Waldenström (M). The γ-globulin peaks are on the left side, and the gray areas correspond to P.A.S. staining.

primary and secondary macroglobulinemia. Two of the patients with primary macroglobulinemia (cases I and II) exhibited an increase in the content of macroglobulin (S ≧ 13.6) to more than 14 %. In one patient (case III) about 50 % of the macroglobulin was of S 12 type. According to Imhof et al. (5) the first two patients have proved macroglobulinemia. Case III has been included in the group because clinical findings support the diagnosis macroglobulinemia Waldenström. The patients with secondary macroglobulinemia (case IV and V) were included in the study on the basis of immuno-electrophoretic demonstration of a high content of β_{2M}-macroglobulin.

Electrophoretic diagram of serum from one of the patients (case III) and from a normal subject are shown in figs. 2 and 3. In contrast to the rabbit sera, these pathological human sera did not reveal separate peaks of P.A.S.-positive material between the β- and γ-globulins, nor was any shoulder-formation seen on the fastest migrating γ-globulin (14).

It is interesting, however, that in the three cases with primary macroglobulinemia a significant increase in mobility of γ-globulin was noted. Case IV and case V exhibited a normal γ-globulin mobility in serum electrophoresis. The increase in electrophoretic mobility of the γ-globulin protein fraction (cases I, II and III) may be due to increased glycoprotein content and in this respect be related to the be

Table I. Ultracentrifuge and immuno-electrophoretic analyses in patients with primary and secondary macroglobulinemia (group I and 2). Cases no. I—III were suffering from primary and cases no. IV and V from secondary macroglobulinemia

Case	Age	Sex	Sedimentation constant (macroglobulin)	Amount macroglobulin %	Immunoelectrophoresis
I	69	♂	17.3 S	15.	β₂M-paraprotein
			22. S	5.2	
II	0	♂	13.6 S	16.7	β₂M paraprotein
			0.3 S	.8	
III	41	♂	1— S	6	β₂M-paraprotein
			16.0 S	1.9	
			17.5 S	1.4	
			19.3 S	.9	
IV	5		14.6 S	9.6	Increase of β₂M
			25.6 S	5.8	
V	60		15.6 S	1.4	Increase of β₂M

Of the total serum protein content.

The rinsing in sulfosalicylic acid does not result in any loss of protein from the strips as protein stained strips after this treatment could be restained with amido black and thereby exhibit a protein pattern identical to that obtained before rinsing. Similarly the protein staining procedure with subsequent rinsing does not alter the intensity of the P.A.S. stain when this is compared to the intensity of the same stain applied without previous protein staining.

The reverse sequence of staining operations however cannot be used. Strips stained with P.A.S. are easily bleached in 0.5 % KOH in 99 % ethanol. It is therefore easy to eliminate the P.A.S. stain in strips after they have been stained for protein. It was noted that if the P.A.S. staining is done before the protein staining a loss of 40—50 % of the initial protein-staining intensity of the serum albumin is seen. This loss was not due to the exposure to the alkaline ethanol (10

B. Application of the double staining method to blood serum from rabbits and patients

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secondary macroglobulinemia exhibited normal mobility of γ-globulin.

Application of the method to serum from patients suffering from rheumatoid arthritis with positive rheumatic factor reactions did not indicate any alteration which could reasonably be ascribed to a change in the macroglobulin content

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Table II Laboratory findings in patients with rheumatoid arthritis (group 3)

Case	Age	Sex	Hb	ESR (mm/hr)	Sheep cell aggl.	Latex fix. test	LE cell test	Severity[1]
1	25	♀	61—77	56—17	+ 80	+	−	I
	73	♀	90—80	70—70	−	+	−	II
3	51	♂	93	26—4[illegible]	+ 2,560	++	−	II
4	63	♀	85—90	60—58	+ 160	(+)	+	II
5	77	♀	70—70	105—103	+ 640	++	−	III
6	68	♀	68—74	101—75	+ 80	+	−	III
7	51	♀	107—77	70—7[illegible]	+ 1,280	+		III
8	71	♀	83—100	82—52	+ 80	+		III
9	51	♀	101—85	31—40	+ 80	+	−	III
10	54	♀	0—93	89—54	+ 160	++	−	III
11	50	♀	73—84	37—51	+ 2,560	+		IV
1	64	♀	73—73	7[illegible]—116	+	(+)	−	IV
13	54	♂	104	28	+ 320	+	−	IV
14	69	♀	94—77	100	+ 160	++	−	IV

[1] Clinical severity of the disease graduated from degree I to IV

haviour of the paraproteins found in some cases of myelomatosis (12 13 15)

Recent studies on the nature of rheumatic factor (RF) have justified the assumption that RF is a macroglobulin of the S 19 type with a carbohydrate content of about 10 , (9 16 17) Further similarities between RF and macroglobulins have been shown serologically by Hammack and Holley (3) and by Kritzman et al (8) It was therefore investigated whether blood serum from patients with rheumatoid arthritis with a proved content of RF would show any sign of this macroglobulin factor when studied with the double-staining electrophoresis method Table II contains the pertinent laboratory and clinical data from these patients. Electrophoretic analysis of the sera from patients with rheumatoid arthritis, however failed to reveal any abnormality of these sera which could reasonably be ascribed to a change in the macroglobulin content. This is no argument against the concept that RF is a macroglobulin or against the fact that RF is increased in the sera The reason why this macroglobulin does not manifest itself may be the minute amount it represents or its close association with the γ-globulins.

Summary

A method has been devised for successive staining of protein with amido black 10 B and of protein-bound carbohydrate with periodic-acid Schiff (P.A.S.) on the same paper electrophoresis strip. Application of this method to blood serum from rabbits treated with injections of casein revealed a protein distribution which accords with an increase of the macroglobulin content demonstrated by means of ultracentrifugation.

The same method applied to sera from three patients with primary and two with secondary macroglobulinemia did not result in the demonstration of any alterations in the protein pattern comparable with the observations in rabbit sera. The electrophoretic mobility of the γ-globulin fraction was increased in the three sera from patients with primary macroglobulinemia. The sera from two patients with

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Pulmonary Blood Volume and its Relation to Pulmonary Hemodynamics in Cardiac Patients

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Resistive properties of the pulmonary vascular bed in human subjects have been extensively studied since the introduction of the heart catheterization technique. Attempts have also been made to quantitate pulmonary blood volume changes during various experimental conditions. Dye dilution technique according to Stewart-Hamilton has been used for needle-to-needle volume estimations. This needle-to-needle volume has usually included either all heart chambers or at least the left heart side. It has therefore been impossible to deduce the site of occurrence of any observed volume changes. It could have been in the pulmonary vascular bed — especially volume-regulating vessels — or in the heart chambers (4, 8, 9, 17).

More exact estimation of the volume in the pulmonary vascular bed has been made possible recently through left atrium catheterization and dye dilution technique with two indicator dyes (3, 13). Transseptal technique has considerably simplified the procedure of left atrium catheterization (1, 2, 16, 18) and thus made the investigation procedure more physiological. Simultaneous study of pulmonary vascular resistance and pulmonary blood volume may help us to arrive at a better understanding of pulmonary hemodynamics and its regulation in health and disease. The purpose of this paper is to present the method used in this laboratory for studies of resistance and capacitance properties of the pulmonary vascular bed, and the results from the first 30 cardiac patients and 2 subjects with normal circulation. For the sake of clarity it may be emphasized that pulmonary blood volume refers to circulating pulmonary blood volume throughout this paper.

Methods

Right heart catheterization was performed in all patients via an antecubital vein. Lehman catheter No. 8 with 4 side holes at the tip and with no end-hole was used to facilitate instantaneous mixing of the injected dye. The left atrium was catheterized percutaneously

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Expired gas was collected in a Douglas bag during determination of cardiac output. The oxygen and carbon dioxide fractions of the expired gas were measured on a Scholander apparatus. Oxygen consumption was calculated and expressed in ml/min. STPD. Ventilation was expressed in l/min. BTPS. Arterial and venous blood samples for the analysis of oxygen saturation were obtained immediately before the injection of the dyes in a limited number of patients. Cardiac output according to Fick could thus be calculated. The oxygen saturation was measured on the Beckman model B spectrophotometer by the method of Nahas (14)

Pulmonary vascular resistance was expressed as $\frac{\bar{P}_{PA} - \bar{P}_{LA}}{Q}$ wherein $\bar{P}_{PA}$ and $\bar{P}_{LA}$ represent mean pressure in mm Hg in pulmonary artery and left atrium respectively and Q represents cardiac output l/min.

Procedure

Investigation was performed in the morning. The patient was in the postabsorptive state lying comfortably on the X-ray table. The right heart catheter was placed in the main stem of the pulmonary artery and then the transseptal catheterization was performed, the catheter being placed in the left atrium.

After the insertion of the intraarterial catheter the patient was allowed to rest for about 20 min. before cardiac output measurement was started. Intravascular and intracardiac blood pressures were recorded simultaneously and immediately before and after the measurement of cardiac output. In the majority of patients another set of blood flow and pressure measurements was performed 20 min. later to study the effect of some physiologic or pharmacologic stimuli. In limited number of patients this procedure could be repeated as many as 4 times without discomfort to the subject (15 21)

Five subjects are excluded from the study because of the error in the collection of the dye.

Material

Selected clinical and hemodynamic data are presented in table I Sixteen male subjects ages 18 to 75 years and 16 female subjects, ages 29 to 62 years, were investigated. Distribution of patients with respect to diagnosis was as follows: aortic valvular disease: 7 patients mitral valvular disease: 10 combined aortic and mitral valvular disease 7; systemic arterial hypertension 1; cardiomyopathy 1; constrictive pericarditis 1; coarctation of the aorta 3 and no cardio-vascular abnormality was found in 2 subjects. Severity of the cardiac disease with respect to function varied between functional groups I and IV according to the classification of the New York Heart Association. 11 patients had atrial fibrillation. 18 patients were on continuous digitalis therapy

Results

Values for pulmonary blood volume and selected hemodynamic data are presented in table I

Pulmonary blood volume varied between 800 and 200 ml with a mean value of 545 ml. The mean pulmonary blood volume corrected for body surface area was 310 ml/sqm BSA with variation between 450 ml/sqm BSA and 180 ml/sqm BSA, except in one patient who had 126 ml/sqm body surface area. Fig 3 shows pulmonary blood volume/m BSA distribution with respect to the severity of cardiac dysfunction. On the average the pulmonary blood volume tends to decrease with increasing functional disability leveling off in functional group III where the values are considerably scattered. Pulmonary blood volume is scattered over a wide range of values in patients with atrial fibrillation, most of them belonging to functional groups III and IV Lowest values for pulmonary blood volume (below 250 ml/sqm BSA) were recorded in 5 patients all belonging to functional groups III and IV Three of these 5 patients had atrial fibrillation. Only 5 out of 25 patients in functional groups II to IV had pulmonary blood volume higher

using a transseptal needle (16) The catheter which replaced the needle in the left atrium had the same length and the same intraluminal volume as the catheter in pulmonary artery The end of this catheter was open and there were side holes within 2 cm of the tip of the catheter A polyethylene catheter (PE 20) was placed in the brachial artery in most cases and a short catheter of the same intraluminal size as the left atrial catheter was placed in the femoral artery in the remaining patients. The insertion of the intraarterial catheters was done percutaneously Intravascular blood pressures were measured using Elema pressure transducers with electromanometers. The recording unit was a 6-channel Elema photographic recorder (Elmquist) All electromanometers were repeatedly calibrated against the same mercury or water standards. The reference level for all pressure measurements was a point 5 cm below the sternal notch of the supine patient.

Bromsulphthalein (12 22) and Cardio-Green (6) were used as indicator dyes for blood flow and blood volume determinations. The pre-determined amounts of the two dyes were injected simultaneously and instantaneously into the pulmonary artery and the left atrium respectively The dye remaining in the dead-space consisting mainly of the catheter volume was always withdrawn 1—2 sec after each injection and analyzed for dye concentration This amount of dye was then subtracted from the total dye amount in the present syringe The injection site for each dye was chosen instantaneously one into the pulmonary artery and the other into the left atrium with respect to different subjects. It was constant in the same subject during different experimental procedures. All dye samples were collected during either 1 sec or 2 sec time intervals (depending on the assumed length of mean transit time) from the arterial catheter using a rotary fraction collector Approximately 40 ml of blood were required for each collection. The extinction of each dye in the plasma of each sample was read on a Beckman model B spectrophotometer Calibration curves for bromsulphthalein were constructed according to Wassén (22) Water and saline were used to construct calibration curves for Cardio-Green in the first part of the study Human plasma replaced saline and water in the last part of the study

The results were virtually unaffected by these changes in method. The detailed description of the Cardio-Green analysis and discussion of the methodological modifications will be published elsewhere

The average of the two cardiac output measurements as calculated from the bromsulphthalein curve and from the Cardio-Green curve was used for volume calculations. In four instances only one of the two dye dilution curves could be used for cardiac output calculations due to technical error injecting the dye. Mean transit time could, however be calculated from both curves because the error in the injected amount of the dye does not involve an error in the calculations of mean transit time for that indicator

Cardiac output and mean transit time were calculated from each dye curve according to the usual Stewart Hamilton formula (7,20

$$\dot{Q} = \frac{(60)\,I}{(\Sigma\,\text{Conc})\left(1-\frac{\text{Hct}}{100}\right)\text{Ti}}$$

$$\text{MTT} = \frac{\Sigma(\text{Conc.} \times \text{time})}{\Sigma\,\text{conc.}}$$

where

$\dot{Q}$ = cardiac output (l/min.)

MTT = mean transit time (sec.)

I = amount of indicator injected

Σ Conc. = sum of the concentrations read at 1-second intervals from the indicator dilution curve, extrapolated through 3 logarithmic cycles

Time = time corresponding to each concentration, in seconds, from the moment of injection

Ti = time interval between each sample

Hct = hematocrit.

The following volume calculations were carried out

PA → Art volume = $\dot{Q}$ (ml/sec.) × MTT PA → Art (sec.)

LA → Art volume = $\dot{Q}$ (ml/sec.) × MTT LA → Art (sec.)

Pulmonary blood volume (Q) = $\dot{Q}$ (ml/sec.) × (MTT PA → Art — MTT LA → Art) (sec.)

(PA = pulmonary artery Art = systemic artery LA = left atrium)

Pressures mm Hg					R_{pulm}, mm Hg / l/min	Q l/min	MTT_{pulm} sec	Q_{pulm} ml	Q_{pulm} / m² BSA ml	SV ml
P_{RV}		$\bar{P}_{PA}$	$\bar{P}_{LA}$	$\bar{P}_{ART}$						
S	D									
19	2	11	2	79	0.99	8.09	3.82	515	312	100
30	6	18	10	87	1.39	6.46	7.84	822	440	113
26	3	13	8	71	0.95	5.25	3.92	518	316	94
98	14	60	23	74	10.54	3.51	9.24	540	338	36
51	10	36	18	150	4.83	3.79	10.16	642	333	63
—	—	11	8	85	0.50	5.96	6.29	625	349	78
—	—	16	—	142	—	6.00	3.81	581	266	64
28	—	15	18	82	0.79	7.16	4.83	577	337	72
97	7	55	29	143	8.93	2.91	11.86	575	383	23
	—	52	41	123	4.53	3.07	5.33	284	187	27
26	15	17	11	79	1.37	4.39	8.04	588	313	35
35	—	25	15	76	2.30	4.34	8.03	581	325	64
30	8	37	22	79	5.12	2.93	11.83	572	344	39
—	—	12	6	82	0.72	8.37	3.68	513	291	89
26	4	18	8	77	2.76	3.99	4.01	266	172	55
26	2	19	5	109	0.53	9.46	5.04	793	400	133
47	18	27	17	92	2.23	4.44	9.40	696	363	131
18	2	12		94	1.11	6.28	5.26	551	284	91
44	9	27	20	126	1.75	4.01	7.06	472	288	72
25	—	15	7	94	1.31	6.13	5.73	586	327	100
27	3	18	10	91	1.38	5.80	5.97	577	317	98
—	—	9	2	82	1.40	5.72	5.63	538	302	64
12	1	17	10	117	2.42	3.30	3.39	187	126	27
—	—	33	23	89	2.08	4.81	9.85	790	451	69
36	4	29	20	115	2.55	3.47	5.04	293	179	40
—	—	25	17	102	1.43	6.28	4.28	448	257	86
—	—	19	8	110	1.42	8.40	4.14	379	318	93
27	6	21	13	98	0.97	9.25	4.85	748	361	117
—	—	30	22	100	1.49	5.37	6.17	553	327	61
40	6	23	13	71	2.30	4.40	8.47	622	337	50
—	—	39	22	93	3.72	4.57	4.15	316	178	62
—	—	18	4	126	1.43	9.77	4.28	697	359	119
						Mea	6.36	545	310	

Hpt = hypertension Ccl = cardiosclerosis BSA = body surface area; HR = heart rate
mean pulmonary arterial pressure $\bar{P}_{LA}$ = mean left trial pressure $\bar{P}_{ART}$ = mean arterial pressure
transit time Q_{pulm} = pulmonary blood volume SV = stroke volume.

pulmonary vascular resistance. No correlation was found between radiographical heart volume and pulmonary blood volume. Patients with isolated mitral valvular disease did not exhibit greater pulmonary blood volumes as compared with other patients who had no signs of mitral valvular involvement.

Table I

Case no.	Sex	Age	Diagnosis	BSA m	Funct. group	Rtg. heart vol. total ml/ rel. ml/m BSA	Digitalis	HR τ = AF
1	○	43	AoI + (AoS)	1 65	III	660/ 400	+	81
2	♂	45	AoI + AoS + MS	1.87	I	1,290/ 690	+	57
3	○	46	(MI) + MS	1 64	III	900/ 550	+	56
4	○	44	(MI) + MS + (AoI) + AoS + TI	1 60	IV	1 650/1,030	+	97 –
5	○	56	MS + Hpt	1.82	II	1,930/1,060	+	57 –
6	o	33	(AoI) + AoS	1 79	II	750/ 420		76
7	○	43	(AoS)	1 43	II	530/ 370	+	94
8	○	29	MS + AoI + AoS	1 71	II	840/ 490		100
9	○	55	MS + Hpt	1.50	III	1,050/ 700	+	128 +
10	♀	62	Cacl + Hpt	1.52	IV	1,050/ 690	+	115 +
11	o	5	Constr peric	1.88	IV	1,030/ 560	+	127 +
12	o	57	MS (AoI) AoS	1 79	II	750/ 420		68
13	○	58	MI MS	1 68	IV	1 430/ 850	+	73 +
14	o	18	Nihil	1 76	I	510/ 290		94
15	♀	40	AoI AoS	1.55	III	650/ 420	+	73
16	o	24	Nihil	1.99	I	935/ 470		70
17	o	45	Card. myop.	1.92	II	980/ 510	+	34 +
18	♂	8	AoI (AoS)	1 94	I	640/ 330		69
19	♀	47	MS + AoI	1.64	III	970/ 570	+	56 +
0		49	MS	1 79	III	860/ 480	+	61
1	o	25	(AoI) AoS	1.82	II	770/ 400		59
22	o	31	Coarc ao.	1 78	I	630/ 360		83
3		5.	MS	1 48	III	640/ 430	+	123 +
4	o	50	MS (AoS)	1 75	III	1 050/ 620		70
5	♀	5.	MS	1.64	IV	950/ 590	+	87 +
26	○	45	MS AoI AoS	1 74	II	650/ 380	+	73
27	o	0	Coarc ao.	1.82	II	720/ 400		88
8	♂	38	(MI)	2.07	I	860/ 470		79
29		45	MS	1 69	III	630/ 370	+	83 +
30	o	51	AoS (AoI)	1 74	IV	1,230/ 710		83
31	♂	53	MS	1 78	III	760/ 430		74
32	o	22	Coarc ao.	1 94	I	830/ 430		82

AoI = aortic insufficiency AoS = aortic stenosis MS = mitral stenosis TI = tricuspid insufficiency
AF = atrial fibrillation P_{RV} = right ventricular pressure (S = systolic D = diastolic) $\bar{P}_{PA}$ =
$R_{pulm.\ vasc.}$ = pulmonary vascular resistance $\dot{Q}$ = cardiac output $MTT_{pulm.}$ = pulmonary mean

than the average value for patients in functional group I

There were no definite trends in pulmonary blood volume distribution with respect to the degree of the elevation in pulmonary arterial pressures. However a tendency was noted to a decrease in pulmonary blood volume with increasing

tended to be smaller in patients with increasing left atrial pressure. This suggests that the pulmonary blood volume is determined not merely by the outflow resistance from left atrium to left ventricle. This suggestion is furthermore supported by the fact that patients with isolated mitral valvular stenosis did not have a greater mean pulmonary blood volume than patients without mitral valvular involvement.

Absence of relatively large pulmonary blood volume in patients with mitral valvular stenosis in the present study is in conflict with results published by Dock et al (3). The difference between the esults could be due to the difference in the patient material studied. It is always difficult to assess all the diagnostic details in patients with rheumatic valvular lesions and thus make the appropriate comparisons between different patients. Another reason for the observed difference might be a methodologic one. The blood volume of the left trium, which is markedly increased in cases with mitral stenosis and elevated left atrial pressure, may be more completely included in the calculation of blood volume between left atrium and the collecting catheter in the present study. The dye injected through transseptal catheter with side holes is probably better distributed and mixed with the whole blood volume in the left atrium than was the case in the material of Dock et al. Blood volume in the left atrium is thus more completely excluded from calculated pulmonary blood volume.

Fig. 3 which illustrates a tendency for pulmonary blood olume to decrease with increasing cardiac disability supplies a possible explanation for the non-dependence of pulmonary blood volume on outflow resistance in this type of patient material. A more or less cute increase in

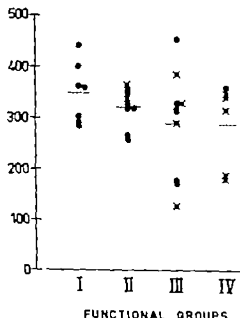


Fig. 3. The distribution of the values for pulmonary blood volume in 22 patients with respect to functional disability. ● indicates patients with atrial fibrillation. Functional groups according to the classification of the New York Heart Association.

pulmonary blood volume due to blood engorgement behind valvular lesions or a failing left ventricle may in the long run be counteracted by forces acting to decrease the pulmonary vascular bed. Forces determining the size of the pulmonary vascular bed may be interstitial edema, structural changes in parenchyma and vascular walls and/or increased vascular tone. The vascular bed becoming less compliant because of the action of these forces may withstand higher intravascular pressures without dilating and increasing its volume. This suggestion is sup-

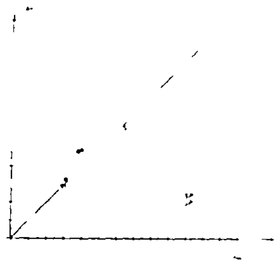


Fig 1 Relationship between the two simultaneously determined cardiac outputs (Q̇) with respect to the injection site (PA Art. = injection into pulmonary artery LA Art. = injection into left atrium) and with respect to the two different dyes used (BSP bromsulphthalein) 49 determinations in 28 patients. The lin is drawn at 45 angle.

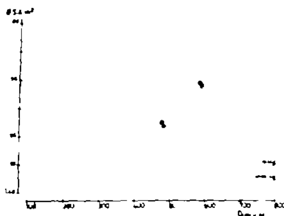


Fig 2 Relationship between the pulmonary blood ol me (Q) and the body surface rea (BSA) in 32 patients. $\bar{P}_{LA}$ mean left trial pressure.

Discussion

Good agreement was found between the two simultaneously determined cardiac outputs with respect both to injection site and to the two different dyes used (fig 1) There was also a close linear correlation between the mean cardiac output determined by dye dilution technique and cardiac output measured using the method of Fick in all cases where all these three determinations were done simultaneously Thus no systematic differences disturb the interpretation of the results

Pulmonary blood volume values recorded in the present study are in good agreement with those presented by Dock et al. (3) and Milnor et al. (13)

Lung size (volume of tissue) is probably one of the critical factors determining the size of pulmonary vascular bed and thus pulmonary blood volume, besides governing the size of airways (11) Within a given group of patients differences in the lung size can be accounted for in terms of differences in body growth and individual variations. Pulmonary blood volume was well correlated to height only in the patient group with normal left atrial pressure (below 13 mm Hg) Pulmonary blood volume values from the patient group with left atrial pressure above 13 mm Hg were scattered and showed no correlation to height. Almost equally good correlation is seen in both groups of patients when the pulmonary blood volume is related to body surface area (fig 2) BSA since it includes weight measurement more closely relates to changes in total blood volume in the cardiac patients with congestive heart failure and elevated left atrial pressure. Pulmonary blood volume being a part of total blood volume it is reasonable to expect a closer correlation between BSA and pulmonary blood volume

There was virtually no correlation between the pulmonary blood volume/m² BSA and the degree of left atrial pressure elevation. If any relationship could be detected between these two parameters it was that the pulmonary blood volume

lier part of this discussion suggests, however that the progressive development of the decrease in pulmonary blood volume, the increase in heart rate and the decrease in stroke volume are the results of the same process in the progressive disease affecting the size of pulmonary vascular bed as well as the function of the heart This suggestion does not exclude the reasonable possibility that pulmonary blood volume is as important for left ventricular output, when the patient is submitted to different physiological stimuli or stress, as is venous return for the output of right ventricle. Under similar circumstances the size of pulmonary blood volume could be influenced acutely by sudden changes in outflow resistance from left atrium to left ventricle as well as by the right ventricular output into pulmonary bed.

In conclusion the present results are in agreement with the statement (10) that the characteristic pattern of pulmonary circulation in patients with heart failure is not the ncreased pulmonary blood volume but the elevation of blood pressures in pulmonary vessels. Pulmonary capillary pressure is sufficiently high to cause the transudation of the fluid into alveoli and interstitium especially n the lower parts of the lungs. Consequent to elevated pulmonary pressures on the pulmonary vascula bed together with interstitial edema, there is impairment of the elastic properties of the lungs. The potential olume of pulmonary vessels becomes restricted.

Summary

Pulmonary blood volume between pulmonary artery and left trium was measured in 32 cardiac patients using two indicator dyes injected into the pulmonary artery and the left atrium respectively

The mean pulmonary blood volume per sqm body surface area corresponds well with values presented previously in the literature where a similar technique was used.

There were no differences in pulmonary blood volume between the patient group with mitral valvular lesion and those who had no involvement of mitral valves.

There was no evidence to suggest that resistance to blood outflow from left atrium to left ventricle determined the pulmonary blood volume in this series of cardiac patients.

The present results suggest that the origin of decreasing pulmonary blood volume in patients with increasing heart rate, of decreasing stroke volume and possibly of increasing pulmonary vascular resistance lies in structural and/or functional changes in the lungs and the heart.

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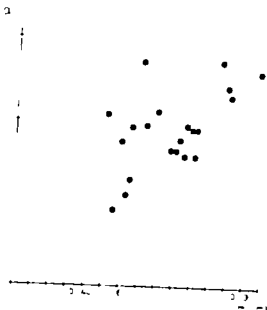


Fig 4 Relationship between the pulmonary blood volume (Q) and the stroke volume in 32 patients. O atrial fibrillation

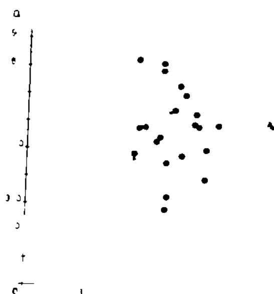


Fig 5. Relationship between the pulmonary blood volume (Q) and the heart rate in 32 patients. O = atrial fibrillation.

ported by the observation that patients with higher pulmonary vascular resistance tended to have a smaller pulmonary blood volume No close correlation between these two parameters is to be expected because in patients with elevated pulmonary pressures the main part of pulmonary blood volume may be located in the most compliant parts of the pulmonary vascular bed i.e. capillaries and veins (5) while relatively less of the pulmonary blood volume may be in the less compliant part of the vascular bed, i.e. arteries and arterioles. However any positive correlation may suggest that both an increase in pulmonary vascular resistance and a decrease in pulmonary blood volume are the results of the same more or less progressive process in the lungs. Increasing radiographic vascular markings in the lungs with increasing cardiac dysfunction may therefore, in comparison with the intravascular circulating blood volume reflect more closely the process, i.e. edema, lymphatic engorgement and structural changes, in the parenchyma of the lungs and in the vessel walls.

Pulmonary blood volume constitutes a blood reservoir on which the output of the left ventricle is dependent in normal circulation (19) Fig 4 illustrates the relationship between pulmonary blood volume and stroke volume, suggesting the existence of direct correlation between the amount of blood in the lungs and the ventricular output per beat in this patient material. No correlation could be found between pulmonary blood volume and cardiac output. Interrelationship between pulmonary blood volume and ventricular action is further illustrated in fig 5 which shows that the patients with increasing heart rates had decreasing pulmonary blood volume. Absence or presence of digitalis medication did not have any influence on this correlation. Similar observations have been described in normal man (19) The present study does not supply enough evidence to explain the exact mechanism behind these interrelationships. The ear

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Hepatic Carcinoma Simulating Hyperparathyroidism

By

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Carcinoma with bone metastases is sometimes combined with disturbances of the calcium-phosphorus equilibrium (2, 3 6 7 21 26—29 34 35 47 50 54) In the majority of cases the metastases are osteolytic, whereas osteoplastic metastases usually do not give any apparent changes of the calcium-phosphorus levels in blood and urine. In a series of 699 patients with metastatic bone destruction (cancer of the prostate not included) 8—9 % of the patients were found to have hypercalcemia (53) In certain forms of cancer such as cancer of the breast, the incidence of hypercalcemia is even higher a percentage of 14—17 having been reported (48 53) The blood phosphorus is usually normal or slightly increased, seldom lowered (21) Isolated reports on an increased calcium excretion through the kidneys have also been published it is supposed that the urinary output reflects the amount of bone tissue engaged (34) Serum alkaline phosphatase is increased especially in cases with osteoplastic metastases (53)

In recent years few cases of hypercalcemia associated with malignancy have been observed without any signs of bone destruction. These cases sometimes have had clinical and laboratory signs identical with those found in hyperparathyroidism. Their parathyroid glands have not shown any signs of hyperfunction at autopsy or surgical exploration, and the cause of the changes is still obscure. We have observed a similar case in the medical department of the University Hospital of Uppsala.

Case report

52-year-old carpenter admitted t the medical clinic in Aug. 1959 Previous history without remarkable features. During the past few months he had sometimes observed dark urine and swollen ankles. H had lost his appetit and complained of increasing fatigue. His relatives had noticed that he had become somewhat indolent. On physical examination one finds thin, pale man without signs of cardiac insufficiency The abdomen is of normal appearance. H is taciturn, cerebrates rather slowly and is at times almost apathetic. No other remarkable findings.

Laboratory examination. Hb 12 4 g%, WBC normal, N protein or reducing sugar in the urine. The E. S. R. is 46 mm/hour but falls

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a bilirubinemia of 2.0 mg%, pos. flocculation tests, alkaline phosphatase increased as before, low PP 47 %, increased GOT 111 units. Total plasma protein 6.3 g% albumin 2.7 g%, globulin 3.6 g%. X-ray examination of the stomach reveals a somewhat irregular contour at the minor curvature and suspected varices of the esophagus. 5 liters of yellowish ascites containing 1 % protein is removed on laparocentesis. His state deteriorates and the patient is periodically unconscious until he dies two months after the admission.

At *the autopsy* a large liver of 2,280 g is found. In the dorsomedial part of the right lobe there is tumor as big as a child' head. In the rest of the parenchyma multiple smaller tumors are found. The tumors are partly superficially situated and umbilicated. The gall bladder and the larger bile ducts are free. There is also a carcinomatous thrombosis of the hepatic veins extending up into the inferior caval vein and projecting into the right atrium. The carcinomatous thrombosis also continues downwards to the level of the renal veins but leaves these veins free. The stomach is normal, there are varices in the lower part of the esophagus. In the left lung there are 4—5 subpleural pea-sized metastases.

Histopathological examination (Prof. Hultquist) Similar pictures are seen in the large and the small tumors. The tumor tissue consists of pale cells with an alveolar and somewhat papillary arrangement in strikingly well-developed hyalinized and sclerotic stroma with small calcifications (fig. 1) Probably it is form of hepatic cholangiocarcinoma. There are large tumor masses with abundant growth engorged with blood in the inferior caval vein, partly of almost sarcomatous structure Here and there, however changes of the same type as in the hepatic tumor can be seen. In the neighbourhood of the pancreas lymph node with vegetations of adenocarcinoma in the pulp is seen. There are suspected tumor thrombi in small vessels of the pancreas and in the capsules of some lymph nodes. There are bronchopneumoniae and pulmonary metastases. A pulmonary embolus with cancer vegetations is found. The organs of the neck and mediastinum are carefully examined. No parathyroid glands are discovered. No metastases to the bone system are found.



Fig 1. Photomicrographs of the tumor t two different degrees of magnification. (Courtesy of Prof. G. Hultquist.) Note papillary and "semiglandular" arrangement of cancer cells.

Discussion

This case of cholangiocarcinoma without skeletal metastases and without signs of parathyroid hypertrophy or adenoma presented the laboratory characteristics of hyperparathyroidism hypercalcemia, hypophosphatemia, hypercalcuria, increased serum alkaline phosphatase increased renal phosphate clearance and lowered TRP

Similar cases simulating hyperparathyroidism in malignancy without skeletal involvement are rare. Gutman et al. (21) in 1936 reported a case of bronchogenic carcinoma without metastases and with a localized and relatively inactive Paget's disease in the femur of a man, 57 years old. H had an inexplicable hypercalcemia and a low-normal blood phosphorus, the bone system and the

Table I Blood le els and urinary excretion of calcium and phosphorus during the time of observation

Date	Blood			Urine				Comments
	Calcium (mg)	Phosphorus (mg)	Alk. phosph. B. L. units	Calcium (mg/ 24 hrs)	Phosphorus (mg/ 24 hrs)	Phosph. clear (ml/ min.)	TRP	
Aug								
14	11.2	3.4	9.9	—	—	—	—	—
17	—	—	9 7	—	—	—	—	—
27	—	—	6.5	—	—	—	—	—
Sept								
1	—	—	10 4	—	—	—	—	—
3	11.8	3.0	—	400	458	—	—	Ordinary diet
6	—	—	—	364	688	—	—	Ordinary diet
7	12.6	2.9	—	—	—	—	—	—
11	11.8	2.7	—	364	597	—	—	200 mg calc./24 hrs
15	—	2.2	—	—	—	20.6	74	200 mg calc./24 hrs
21	12.8	2.6	—	—	—	—	—	—
23	—	—	—	—	—	—	—	Surgical exploration
26	13.2	3.6	—	—	—	—	—	—
28	11.8	2.3	—	—	—	—	—	—
Oct.								
6	10.6	_5	9 7	254	63	—	—	Ordinary diet
12	13.0	2.2	10.4	264	190	—	—	200 mg calc./24 hrs
14	—	2.4	8.2	—	—	17 0	74	200 mg calc./24 hrs

to 20 mm/hour in a few days. Alkaline phosphatase 9 9 B. L. units (normal value in adults 0 9—2 5) bilirubin 0 6 mg% flocculation tests normal. Prothrombin-proconvertin index 98 On repeated examinations a hypercalcemia of 11—13 mg% is found, the serum phosphorus being low or normal 2 2 —3 4 mg% (table I) On an ordinary diet the 24-hour urinary excretion of calcium is 364—400 mg and of phosphorus 458—688 mg After a week on a low phosphorus-low calcium diet (less than 200 mg calcium/24 hours) the 24-hour urinary calcium excretion is 264 mg the phosphorus excretion 597 mg Renal phosphate clearance is 20 6 ml/min tubular reabsorption of phosphate (TRP) 74 % No signs of hypercalcemia in ECG Good renal function endogenous creatinine clearance 73—80 ml/min., serum creatinine 0.8—1.2 mg% urine concentration to spec gravity 1 024 Total plasma protein 7 4 g%

albumin 3.9 g% globulin 3.5 g% Serum citric acid 32 μg/ml. Fecal fat excretion normal. X ray examination of the bone system shows normal conditions. Intravenous pyelogram and peroral cholecystography are normal. On the suspicion of hyperparathyroidism the patient is accepted for surgical exploration (Prof Hultén) At the operation the neck between esophagus and trachea and anterior and posterior mediastinum is carefully examined. Nowhere any findings of parathyroid adenomas. Parts of the thyroid gland are removed but no parathyroid glands are found. The patient is transferred to the medical clinic again after a few weeks. He is now in a worse state gets hydrothorax, edema of the legs, ascites, visible veins on the abdomen and lower part of the chest. Persisting hypercalcemia and hypophosphatemia, increased renal phosphate clearance and low TRP Signs of impaired liver function appear with

tissue. No details about the parathyroid glands were given. Abouav et al. (1) related a case of masculinizing hypernephroid tumor of the ovary in a 46-year-old woman. She had hypercalcemia, hypophosphatemia, hypercalcuria and hypophosphaturia. After removal of the tumor the blood and urine chemistry became normal. Gold et al. (20) reported a case of hypercalcemia in a 49-year-old man. He had a bronchogenic carcinoma with large hepatic metastases without involvement of the bone system and with normal parathyroid glands at autopsy Lucas (31) described a case of adenocarcinoma of the pancreas with widespread abdominal metastases in a 49-year-old man. He had hypercalcemia, hypophosphatemia, increased serum alkaline phosphatase and hypercalcuria. No parathyroid adenoma was found at operation. Hed (22) reported a case of a malignant tumor of the adrenal medulla with hypercalcemia and uremia in a 60-year-old man. He had normal parathyroid glands and no skeletal metastases were found at autopsy Recently David et al. (17) described a case of clear cell carcinoma of the kidney in a 57 year-old man with hypercalcemia and hypophosphatemia. At autopsy he was found to have liver and lung metastases and normal parathyroid glands.

Cope et al. (14 16) collected cases operated on the presumptive diagnosis of hyperparathyroidism at the Massachusetts General Hospital. They found 248 cases of primary hyperparathyroidism and 51 cases with an erroneous diagnosis. In the latter group there were three patients with malignancy and normal parathyroid glands one with a bladder tumor and two with renal carcinoma. They had no demonstrable skeletal metastases. The serum calcium returned to a normal level after operation no further details were given, however

Thus, there are about 40 cases reported of this syndrome similar to hyperparathyroidism and associated with malignant disease without skeletal involvement and without hypertrophy or adenoma of the parathyroid glands. Some of these cases are difficult to evaluate critically for instance where no details are given (16 18, 36) Cases without skeletal metastases verified only by X-ray examination (26 27 36) are also difficult to judge. This was demonstrated, for example, in a study of 650 consecutive cancer patients (6) where 10 % had had a positive bone marrow aspiration but 37 % of these had had a negative skeletal X-ray picture. Skeletal involvement in cases of multiple myeloma and malignant lymphoma is also very difficult to exclude due to the disseminated nature of the disease (33, 43)

There remain, however 23 cases (including our own) where the parathyroid glands and the bone system have been examined at operation or autopsy 15 are men and 8 women. The age of the patients has varied from 46 to 76 years and does not differ significantly from the usual age range of patients with malignant disease. The syndrome seems to be especially associated with some particular kinds of cancer Seven cases had bronchogenic carcinoma and six cases had renal carcinoma, at least five of the latter of the clear cell type.

The cause of the changes in blood and urine is obscure. It has been suggested that the hypercalcemia is due to excessive bone resorption resulting from substances introduced into the circulation by the tumor (15) Albright and Reifenstein related a case with hypernephroma and a large metastasis to the sacrum and

parathyroid glands were normal on post mortem examination The disturbances of the blood were considered to be related not to the inactive Paget s disease but to complicating factors as yet wholly obscure Another case was reported from the Massachusetts General Hospital in 1953 (12) a 74-year-old man with a small localized carcinoma of the prostate and a huge hemangiosarcoma of the liver The bone system was normal at autopsy The patient had hypercalcemia normal or slightly increased blood phosphorus and increased alkaline phosphatase He died in uremia Connor et al. described two cases of bronchogenic carcinoma in two men 62 and 69 years old respectively (15) These patients had hypercalcemia and a lowered serum phosphorus. After operation the calcium and phosphorus values rapidly returned to normal In one of the cases the disturbances came back after recurrence of the tumor Plimpton et al collected ten cases with hypercalcemia and normal or slightly lowered serum phosphorus in malignant disease without bone destruction (35) In three of the cases the removal of the tumor was followed by a rapid fall of the serum calcium In one of these a recurrence of the tumor was again associated with hypercalcemia Three of the cases were renal carcinomas of the clear cell type one was a renal carcinoma with a combination of clear cells and anaplastic features Two patients had bronchogenic carcinoma one of these also had a reticulum-cell sarcoma of the spleen Two patients had endometrial carcinoma and one patient had a papillary adenocarcinoma of the ovary The tenth patient had Hodgkin s disease. The authors found no signs of parathyroid hypertrophy or adenoma at operation in two patients or at autopsy in seven patients. Laird Myers (26, 27) found some cases of hypercalcemia in association with malignancy from the kidney the uterus, the urinary bladder the breast, and with malignant lymphoma These cases had no signs of skeletal involvement on X ray examination. No further details were given. From the Massachusetts General Hospital (13) a case was reported in 1957 of a man with a squamous-cell carcinoma of the lung with hypercalcemia, slightly lowered serum phosporus and hypercalcuria. No skeletal metastases were found at autopsy but there were signs of osteoporosis. The finding of secondary hyperplasia of the parathyroid glands makes this case a little obscure, however The hyperplasia was considered to depend on a factor from the lung stimulating the parathyroid glands. Schatten et al. (38) related a case of squamous-cell carcinoma of the vulva without skeletal involvement but with metastases to the inguinal region in a 72 year-old woman. The patient had hypercalcemia, hypophosphatemia and hypercalcuria simulating hyperparathyroidism Three normal parathyroid glands were removed without any apparent effect. Only when the metastases were removed did the blood changes disappear but they came back with the recurrence of the tumor No skeletal involvement was found at autopsy Reiss et al. (36) found ten cases of hypercalcemia in association with malignancy Three of them had no signs of skeletal involvement on X ray examination The first case was a carcinoma of undetermined origin with hepatic metastases, hypercalcemia, low TRP the second a carcinoma of the lung with hypercalcemia low TRP and the third a case of chronic lymphocytic leukemia. All these cases had a widespread visceral involvement of tumor

Another effect probably of hormonal nature, is the polycythemia found in some cases of renal carcinoma and bronchogenic carcinoma and also in a few cases of tumors of the female genital tract.

In reality it is perhaps not so strange that malignant cells, especially of epithelial origin, may turn off from their "natural" course and start to produce substances of a hormone-like activity. Nor is it in fact surprising that the effects of these substances essentially mimic the action of hormones of protein or polypeptide nature, since most glandular and mucous epithelium normally has just that power of synthesizing protein material.

Summary

A case of hepatic cholangiocarcinoma is reported. The case presented the laboratory characteristics of hyperparathyroidism. There were no signs of skeletal metastases. No parathyroid adenoma or hypertrophy was found. Similar cases with a "false" hyperparathyroidism reported in the literature are reviewed. Possible mechanisms are discussed. It seems likely that malignant cells of non-endocrine origin sometimes produce substances with hormone-like activities.

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the ilium (3 11) The patient had hypercalcemia hypophosphatemia, hypercalcuria and normal serum alkaline phosphatase The parathyroid glands were normal After irradiation of the metastasis there was a transient normalization of the serum calcium and phosphorus levels. A parathyroid hormone like substance produced by the tumor was proposed A transient fall of the serum calcium was also observed after removal of the malignant tumor in some cases (1 14 15 18 35 38) Histologically there is no close similarity between the different tumors. However in many cases there has been an adenomatous growth or a "secreting type of cells. It is thus possible that the tumor may produce a substance with hormone activity The findings of hypercalcemia and hypophosphatemia in the absence of skeletal metastases in rabbits transplanted with V 2-carcinoma may support this hypothesis (52) It is also known that even normal tissue contains substances with a parathyroid hormone-like action Thus Stewart et al (45) have shown that extracts of spleen and thymus may affect urine phosphate excretion in a way similar to parathormone. In one case (38) an extract from the tumor was actually tested for a parathyroid activity but with a negative result

Another hypothesis is that the tumors may produce a substance stimulating the parathyroid glands (13 46) This possibility however seems much less likely as in the majority of cases small or normal parathyroid glands were found Only in one case there is a report of "secondary hypertrophy of the glands (13)

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From the First Department of Medicine (Head: P. I. Halonen, M. D.), University of Helsinki and from the Second Department of Medicine (Head: I. Vartiainen, M. D.), University of Helsinki and from the Finnish Red Cross Blood Transfusion Service (Head: H. R. Nevanlinna, M. D.), Helsinki, Finland

Inherited Agammaglobulinemia with Malabsorption and Marked Alterations in the Gastrointestinal Mucosa

By

R. Pelkonen, M. Siurala and P. Vuopio

Sprue-like syndromes have been described in connection with hypo- or agammaglobulinemia (4, 11, 14, 16, 19). Only five cases have been reported so far (4, 11, 14, 16, 19) and no data are available on the anatomical state of the gastrointestinal mucosa in these patients. Moreover, it should be noted, that signs of intestinal malabsorption have been found only in the primary acquired form of agammaglobulinemia. This report concerns a case of inherited agammaglobulinemia with intestinal malabsorption, in which multiple mucosal biopsies disclosed severe regressive changes in the whole gastrointestinal tract.

Case report

Case II—3. The patient was a 33-year-old laborer. Up to the age of 15 he had been quite well, with no special susceptibility to infection.

1944 he had scarlet fever complicated by mastoiditis. Left mastoidectomy was performed. One year later a large phlegmon occurred in the left thigh, resulting in chronic edema in the left lower limb. In 1957 he complained of colicky pains in the back and one year later a small stone passed in the urine. Right renal and left ureteral stones were found by intravenous urography.

In 1959 he was admitted to the local T.B. sanatorium for lobar pneumonia in the right middle lobe. Bronchography revealed bilateral bronchiectasis. In 1959—1962 he was treated several times for chronic sinusitis affecting all sinuses. In 1960 the Luck-Caldwell operation was performed without success.

Since 1948 the patient had suffered from attacks of diarrhea lasting from two weeks to three months. During the attacks he had fever, lost weight and became anemic. The stools were watery and bloodstained. In 1959 an explorative laparotomy was performed but nothing specific was found. Microscopic examination of a mesenteric lymph node revealed scanty lymphoid tissue with marked proliferation of the reticular elements.

Since Dec. 1961 the diarrhea had been chronic, with foul-smelling, glistening, bulky stools.

On the patient's admission to this hospital on March 15th, 1962, physical examination

Accepted for publication October 22, 1962.

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X-ray studies

X-ray films of the chest revealed slight fibrosis at the bases. Colography showed a total flattening of the haustra. X-ray examination of the stomach and the small intestine, however disclosed no abnormalities.

Gammaglobulin studies

With immunoelectrophoresis there was almost complete absence of all immunoglobulins (β_2A, β_2M and γ) (fig. 1). The γ-globulin level in paper electrophoresis was 0.16 g %.

Serological studies

The patient' blood group was A_1Rh positive. Only traces of anti-B isohemagglutinins were found. An attempt to immunize with blood group B substance failed to raise the titer. Immunization against paratyphoid-typhoid antigens gave no immunological response. One year earlier the patient had had complete Salk polio vaccination. Now however, the neutralizing antibodies (type I and III) were absent. The Mantoux tests were negative to 1 100 old tuberculin. The antistreptolysin 0 titer was low below 32 U., the heterological antibody titer for cold agglutinins was low normal (1 7). The sheep red cell agglutination titer for rheumatoid arthritis was also below normal limits.

Histological studies of the gastrointestinal canal

Three biopsy (5) specimens were obtained from the *body of the stomach*. No normal body glands were visible in any of the specimens. They were replaced by intestinal tubules. One group of "pseudopyloric" tubules was visible. There was considerable lymphocyte infiltration only few cells resembling plasma cells were seen (fig. 2).

The mucosa of the distal portion of the duodenum revealed marked alterations. The villi were absent, the nuclei of epithelial cells differed in size form and location, the cell borders were only locally discernable, and the striated border low and disorganized. The number of lymphocytes was increased. There were numerous eosinophils. Only a few plasma cell-like cells were seen, but could not, however be definitely proved to be plasma cells (fig. 3).

The sigmoid mucosa was somewhat flattened and revealed considerable degree of tubular disorganization. The number of striated border cells seemed to have decreased. There was cystic dilatation of the bottom of the crypts. Abundant lymphocyte infiltration was present in the submucosal layers (fig. 4).



Fig. 3. The patient. Distal duodenal mucosa. Mucosa is flattened, with total loss of villi. Marked alterations of the striated border cells. Increased lymphocyte infiltration. A few plasma cell-like cells present. Hematox.-eos. 80.

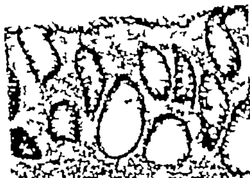


Fig. 4. The patient. Sigmoid mucosa. Flattened mucosa with moderate lymphocyte infiltration, particularly in the submucosa. Increase of the number of goblet cells. Marked alterations in the striated border cells. Dilatation of the crypts. Hematox.-eos. 160.

Family history

All the members of the patient family and the siblings of his mother still living were examined. The γ-globulin levels estimated by paper electrophoresis, the findings in immunoelectrophoresis and the isohemagglutinin titers are given below (fig. 5).

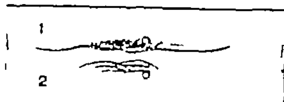


Fig 1 Immunoelectrophoresis. Only a trace of γ-globulin is visible β_2-A and β_2-M-globulins are absent. 1) normal 2) the patient (case II—3)

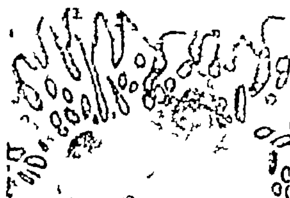


Fig The patient Gastric body mucosa. The normal body glands are replaced by intestinal tubules. Moderate inflammatory cell infiltration by lymphocytes. Only a few cells resembling plasma cells are discernible Hematox.-eos. × 80.

revealed a chronically ill young man of an asthenic constitution, 172 cm tall and weighing 52 kg He was pale and subfebrile The subcutis was markedly reduced. The tongue showed papillary atrophy *The left lower limb* showed pitting edema. Small lymph nodes were palpated in the neck and axillary regions. The spleen and liver were normal in size. The lungs were clear except for a few dry rales in both bases. On sigmoidoscopic examination the mucous membrane was normal.

Hb was 7 7 g red cells 2 66 millions and the hematocrit 27 vol The white cell count was 2,200 with 0 5 myeloblasts, 1 5 promyelocytes, 2 myelocytes, 57 % neutrophils, 4 5 eosinophils, 3.5 % monocytes, 31 % lymphocytes, and 0.5 erythroblasts. The reticulocyte count was 1 4 and the platelet count 142,000

The bilirubin was 0.27 mg % the iron 40 μg % and the total iron binding capacity 190 μg / The plasma total protein was 4 4 and the prothrombin content was normal. The calcium was 8.3 mg % the potassium 4.0 mEq/l and the sodium 139 mEq/l. The cholesterol was 153 mg % and the creatinine 0.9 mg The extinction value in the thymol turbidity test was 0 004 and in the $ZnSO_4$ test 0 005 No turbidity was seen in the *Stolte* reaction. The serum activity of alkaline phosphatase was 5 4 King Armstrong U and of lactic acid dehydrogenase 625 Wroblewsky U

A sternal marrow aspirate revealed marked erythroid hyperplasia with numerous megaloblasts. The almost complete absence of plasma cells was *striking we could find only two* plasma cells in the two microscopic preparations.

Examination of the urine revealed no abnormalities. The stools contained fat 23.7 of the dry weight.

There was no uropepsin excretion in the urine nor any *hydrochloric* acid excretion in the stomach after Histalog stimulation (0.02/l kg of body weight)

The urinary excretion of 5-hydroxy indolacetic acid was 6 9 mg in 24 hours. Paper chromatography of the urinary amino acids was normal. The Figlu test (15 g histidine monohydrochloride daily per os) revealed a slightly increased excretion of formiminoglutamic acid (40 μg/ml) suggesting a deficiency of folic acid.

The chromosome pattern was normal (examined by A. de la Chapelle M. D., Minerva Foundation Institute for Medical Research)

Absorption studies

Peroral glucose tolerance test (1 g glucose per kg body weight) The fasting blood glucose was 71 mg the maximum value encounted after one hour was 108 mg .

d-Xylose (25 g *d* Xylose per os) *The* urinary excretion of *d* Xylose was 2.5 g in 5 *hours.*

Vitamin A (350 000 IU of oily vitamin A per os) The fasting level was 25 μg % and the maximum concentration after ingestion of vitamin A was 51 μg %

Vitamin B_{12} *In the ordinary Schilling test* the urinary excretion of radioactivity was 0.7 % of the given dose There was no increase of radioactivity after addition of the intrinsic factor

The presence of malabsorption was evident from the defective absorption of glucose, d-xylose, vitamin A, folic acid and particularly of vitamin B_{12}. The stools contained considerable amounts of fat and the serum calcium level was below the normal range. The main clinical features were recurring infections, bouts of diarrhea, and more recently macrocytic megaloblastic anemia. The latter was presumably caused by deficient intestinal absorption of vitamin B_{12}, since no increase in the urinary radioactivity occurred after the addition of intrinsic factor. However an additional decrease of intrinsic factor excretion from the stomach should also be taken into account because of the severe alterations in the gastric body mucosa.

The microscopical changes in the gastrointestinal mucosa were striking. In the stomach the normal body glands were replaced by intestinal epithelium. Intestinal mucosa showed alterations similar to those described in celiac disease and the regressive changes in the sigmoid mucosa were distinct, though less striking.

The causal relationship between the intestinal signs and the hypogammaglobulinemia cannot be established definitely. Assuming that this is not a question of mere coincidence, three possibilities should be considered: firstly the mucosal changes are primary and have led to leakage of gammaglobulins into the gastrointestinal lumen (3); secondly the gammaglobulinemia has resulted in atrophy of the gastrointestinal mucosa and thereby to malabsorption; thirdly they are parallel phenomena caused by the same basic defect, which might be hereditary in nature because the primary malabsorption syndrome is also in part heredity linked.

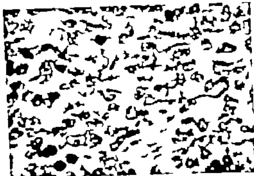


Fig. 6. The patient. Cervical lymph node. Diffuse proliferation of reticular cells. No plasma cells. The lymphocytes are few in number. Hematox.-eos. × 550

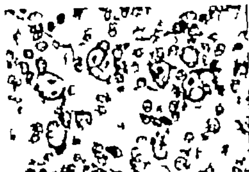


Fig. 7. Cervical lymph node from the identical twin brother of the patient (case II—2). The generally accepted changes suggesting Hodgkin's disease with however only small number of plasma and cells eosinophils. Hematox.-eos. × 550.

Neither the first nor the last possibility can be excluded, although there is no direct evidence for either. The case history suggests that agammaglobulinemia has preceded the occurrence of gastrointestinal symptoms (attacks of diarrhea) and of signs of malabsorption. In addition, the family members suffering from hypogammaglobulinemia showed no signs of malabsorption except perhaps the twin brother of the patient, who had diarrhea and died of malignant reticulosis. These views seem to support

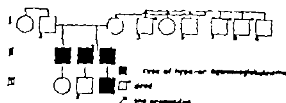


Fig 5 Family pedigree

I—1 Not examined. Healthy

I—2 Born 1902. Father of the patient Healthy Immunoelectrophoresis was normal γ-globulins 0 72 g Anti B isohemagglutinin titer 1 16

I—3 Born 1892 Suffered from pulmonary emphysema since 1947 died at age 68 (1961) of bilateral pneumonia.

I—4 Born 1897 Healthy Immunoelectrophoresis was normal. γ-globulins 1 11 g % Anti A isohemagglutinin titer 1 32

I—5 Born 1899 Healthy Immunoelectrophoresis was normal. γ-globulins 0.87 g % Isohemagglutinin titers Anti-A 1 4 Anti-B 1 4

I—6 Born 1903 Mother of the patient. No specific susceptibility to infections. Examinations revealed a toxic goiter Immunoelectrophoresis was normal. γ-globulins 0.87 g Anti-A isohemagglutinin titer 1 8.

I—7 Born 1909 Since age 14 suffered from pelvic osteomyelitis. Died in 1944 35 years old, with clinical picture of nephrosis, probably due to secondary amyloidosis.

I—8 Born 1911 Died at age of 21 years, probably of pneumonia.

I—9 Born 1913 Healthy Immunoelectrophoresis was normal. γ-globulins 1 11 g Anti-B isohemagglutinin titer 1 8.

II—1 Born 1925 Eldest brother Healthy Immunoelectrophoresis showed slightly decreased γ-globulins, but B_{2}-A and $β_{2}$ M globulins were normal. The γ-globulins (in paper electrophoresis) were slightly decreased, 0.53 g Anti-B isohemagglutinin titer 1 8

II—2 Born 1928. Identical twin brother with the patient Was healthy until May 1959, when he was admitted to a hospital in Sweden because of diarrhea and fever of undulant

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II—3 The patien

III—1 Born 1954
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Anti B isohemagglutin

III—2 Born 1957
(II—2) Healthy but
had infectious mononu
phoresis was normal.
Anti-B isohemagglutin

III—3 Born 1958.
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Discussion

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Professor L. Santesson, M. D., Karolinska Institutet, Stockholm, for placing at our disposal the biopsy specimens from the cervical lymph node. We also express our thanks to Professor E. Saxén, M. D., University of Helsinki, who performed the microscopical examination of the biopsy specimens.

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the second possibility i. e. the primary significance of hypogammaglobulinemia. Hypogammaglobulinemia might have led to decreased resistance of the gastrointestinal mucosa, resulting in repeated attacks of gastroenteritis. This, in turn might have resulted in atrophy of the gastrointestinal mucosa with malabsorption as the consequence. In fact the patient suffered from several bouts of diarrhea before the development of signs of malabsorption

The latter explanation, however deserves a comment. In patients with an unaltered primary malabsorption syndrome as in celiac disease, there are always regressive changes in the intestinal mucosa. However it has not been established that they are an immediate cause of the malabsorption (1) No correlation has been obtained between the clinical picture and the results of laboratory tests, on the one hand and the mucosal changes, on the other hand (7) Moreover the response of the patient s general condition and of the absorptive capacity of the small intestine to treatment did not seem to bear any clear relationship to the histological response of the intestinal mucosa (2 6 15 17) Hence, it is not impossible that the mucosal alterations were secondary to the disturbed metabolism induced by malabsorption e g malabsorption of vitamin B_{12}. On the other hand the present patient differs in some respects from patients with the primary malabsorption syndrome e. g by the intestinalization of the gastric mucosa. Moreover the case history suggests that the signs of malabsorption in particular those of B_1 deficiency have developed in later stages of the disease.

From the above it is evident that no definite conclusions can be drawn although the case history suggests that the agammaglobulinemia might have been the primary disease

The occurrence of renal calculi is also somewhat puzzling The data concerning the calcium metabolism are unfortunately so meager that any discussion of the pathogenesis of the renal calculi in this case should be speculative only

It was interesting to note that histological changes in a cervical lymph node from the patient revealed changes resembling to some degree those found in his twin brother who died of malignant reticulosis (figs. 6 and 7) Wolf (18) suggests that the simultaneous occurrence of hypogammaglobulinemia and malignant reticulosis is not an unexpected one in view of the hypothesis that they may be only different manifestations of a single hereditary mesenchymal defect.

Summary

A case of probably primary congenital agammaglobulinemia with intestinal malabsorption and renal calculi is reported. Multiple suction biopsies revealed marked alterations in the gastric, duodenal and sigmoid mucosa. Three other cases of hypogammaglobulinemia were found in the patient s family one of these, the identical twin brother of the patient, had in addition to the hypogammaglobulinemia malignant reticulosis. The family study disclosed the hereditary sex linked nature of the disease The relationship between the intestinal malabsorption and the agammaglobulinemia is discussed

Acknowledgments

This study was aided by a grant from Sigrid Juselius stiftelse.

W are grateful to the head of the Centrallasarettet, Gävle, Sweden, for giving us the data concerning the patient's twin brother and to

Professor L. Santesson, M.D. Karolinska Institutet, Stockholm, for placing at our disposal the biopsy specimens from the cervical lymph node. We also express our thanks to Professor E. Saxén, M. D., University of Helsinki, who performed the microscopical examination of the biopsy specimens.

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Evaluation of Aqueous Vitamin B_{12} in Long-term Therapy of Pernicious Anaemia

By

Poul Bastrup-Madsen

The investigation presented here is part of a series of papers in which the equivalence of vitamin B_{12} to liver preparations is considered. As there are still clinicians who use liver extract vaguely believing it to contain of an additional factor of importance for the blood formation, it seems justified to bring forward as much evidence as possible that vitamin B_{12} is the only substance lacking in pernicious anaemia, and that liver preparations are active by virtu of their content of this vitamin. One way in which this may be done is to examine whether vitamin B_{12} is equivalent to liver extract in long-term therapy of pernicious anaemia. In the literature few series are available in which patients have been treated for more than two years (2,3 4). On account of this, a series of cases of pernicious anaemia treated with aqueous vitamin B_{12} for from 2 to 10 years, with a mean period of 4 1/2 years, is presented below.

Material

The diagnosis of pernicious anaemia was based on the presence of macrocytic anaemia, megaloblastic bone marrow, histamine-fast achlorhydria and a typical response to anti-pernicious-anaemia therapy. In cases diagnosed after 1956 the presence of low serum vitamin B_{12} values was also included as a diagnostic criterion.

In 21 cases, other forms for anti-pernicious-anaemia therapy had been given before vitamin B_{12}. This therapy had consisted in liver extracts, desiccated hog's stomach preparations and hog pyloric mucosa together with crystalline vitamin B_{12}. Twenty-two patients had only received therapy with vitamin B_{12}. The efficacy of the therapy was evaluated by the same criteria as have always been the standards for the adequacy of liver therapy, i. e. the capability of maintaining an optimal haematological remission and of prohibiting the development of neurological manifestations.

During the entire period of maintenance therapy with vitamin B_{12}, all the patients were regularly seen by the author in our out-patient clinic, where a blood count was performed in connection with each injection, and a neurological examination was performed at least three times a year.

The maintenance dose of vitamin B_{12} was in some cases 60 µg every second week. However in most of the cases varying doses had been used during different periods on account of other experiments going on. In these cases,

Submitted for publication November 5, 1962.

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Table I Long-term therapy with aqueous vitamin B_{12} parenterally. Twenty-one patients who had previously received other anti-pernicious anaemia therapy

Case no	Age at end of study	Duration of previous therapy (yrs)	Previous therapy	Duration of parenteral vit. B_{12} (yrs)	Maintenance dose of vit. B_{12} (μg/weeks)	Haematological values: Before vit. B_{12} therapy: Serum vit. B_{12} (μg/ml)	Hb (g %)	RBC (10^6/μl)	VPC (%)	MCV ($μ^3$)	At end of study: Serum vit. B_{12} (μg/ml)	Hb (g %)	RBC (10^6/μl)	VPC (%)	MCV ($μ^3$)
1	53	3.5	HS	2	30/1	101	13.8	4.8	51	106	346	15.5	4.9	48	98
2	55	18	HS	5	150/2	—	14.5	4.8	44	92	644	15.2	4.8	44	92
3	68	1	HS	5	60/4	—	14.0	3.8	42	100	256	16.6	5.1	49	96
4	71	2	HS	3	60/2	28	9.0	1.9	28	145	410	14.7	5.0	49	99
5	69	13	HS—L	4	120/2	—	11.5	3.6	37	104	1,224	14.1	4.5	43	95
6	79	3	HS	4	90/2	—	12.6	3.4	39	114	262	13.2	4.2	40	96
7	72	23	HS—L	5	60/1	—	9.6	2.7	52	106	540	14.5	5.1	49	98
8	85	5	HS	3	60/2	—	13.8	3.5	43	124	330	14.4	4.4	44	100
9	70	1	HS	8	60/3	—	11.1	2.4	33	112	214	15.5	5.2	51	98
10	73	1	HS	9	120/2	—	13.2	3.5	40	114	382	13.4	4.5	40	93
11	72	3	HS	5	60/2	40	10.4	2.5	30	120	400	14.2	4.8	46	97
12	76	1	HS	8	60/2	—	13.9	4.4	43	99	430	14.2	4.5	44	97
13	52	11	HS—L	6	60/1	—	12.3	2.8	37	134	385	12.7	4.2	39	94
14	65	5	HS	2	60/4	134	16.5	5.6	49	87	276	14.5	4.7	43	92
15	72	7	HS	3	60/4	22	15.3	4.9	45	94	196	13.9	5.2	47	91
16	75	3	HS	2	60/4	259	16.3	5.5	47	89	355	15.4	4.7	46	99
17	68	18	HS—L	10	500/3	—	13.2	4.8	45	97	316	13.5	4.9	44	91
18	79	6	HS—L	2	60/2	160	9.5	3.4	36	105	201	14.2	4.3	41	93
19	57	9	HS—L	2	60/2	63	16.0	5.2	48	92	130	15.0	4.7	46	97
20	77	1	HS	6	60/4	—	14.2	4.7	41	88	480	14.4	4.3	41	96
21	72	11	HS	8	60/1	—	12.6	2.9	36	123	435	14.0	4.7	45	93

HS = Hog's stomach L = liver extract.

the lowest dosage had been 60 μg every fourth week and the highest 500 μg every week.

Results

Table I includes the patients who had previously received some form of anti-pernicious-anaemia therapy other than injections of crystalline vitamin B_{12} in aqueous solution. It appears that the haematological status was normal at the end of the study. The erythrocyte values were in no case below those obtained by the previous therapy. In fact in 12 of the cases the values had improved during vitamin B_{12} therapy. It must also be added that in none of the cases did neurological complications develop.

Table II includes the cases which had never received a therapy other than injections of aqueous solution of crystalline vitamin B_{12}. *In all these cases, a normal* haematological status was seen at the end of the study. Neurological manifestations *did not develop* in any of the patients.

Table II Long-term therapy with aqueous vitamin B_{12} parenterally. Twenty-two patients who had received only vitamin B_{12}

Case no.	Age end of study	Duration of vit. B_{12} therapy (yrs)	Initial therapy Dose of vit. B_{12}	Maintenance dose of vit. B_{12} (µg/weeks)	Haematological values: Before therapy: Serum vit. B_{12} (µµg/ml)	Hb (g %)	RBC (10^6/µl)	VPC (%)	MCV (µ³)	At end of study: Serum vit. B_{12} (µµg/ml)	Hb (g %)	RBC (10^6/µl)	VPC (%)	MCV (µ³)
	69	3	150 µg × 14	60/2	20	6.0	1.3	21	167	303	14.7	4.9	43	93
2	69	4	150 µg × 11	60/2	47	11.7	2.9	35	123	689	16.5	6.0	54	90
3	74	4	150 µg × 14	60/2	38	8.0	3.7	30	81	330	12.9	4.4	44	89
4	65	3	150 µg × 25	150/2	32	4.2	1.0	31	135	332	14.8	5.1	48	94
5	81	4	150 µg 14	60/2	38	6.3	1.6	21	132	440	11.5	4.1	41	99
6	69	7	60 µg × 27	60/2	—	13.0	2.9	37	126	630	13.7	4.5	43	94
7	70	6	60 µg × 2.	60/1	—	5.1	1.3	27	210	610	13.3	4.7	45	96
8	82	4	60 µg × 14	60/2	—	11.3	3.9	37	95	258	13.9	4.8	45	93
9	48	2	250 µg × 15		30	12.3	3.0	35	118	246	13.5	4.5	42	92
10	47	2	250 µg × 14	Vary-	—	5.2	1.4	16	119	405	12.4	4.4	41	95
11	69	4	500 µg × 22	ing	72	6.3	1.6	19	118	263	14.8	4.8	42	89
12	61	3	250 µg 14	from	59	8.8	3.9	32	83	192	13.3	5.0	49	97
13	63	4	60 µg × 60	60/4	—	11.1	3.1	33	112	286	11.5	4.5	41	93
14	53	3	250 µg 21	to	—	5.5	1.2	16	133	144	15.5	5.1	50	98
15	68	2	250 µg 49	250/2	38	3.5	0.7	11	159	239	11.7	4.0	41	94
16	69	4	500 µg 14	du-	23	7.3	1.4	23	163	247	12.7	4.0	42	106
17	64	4	150 µg 14	ing	20	13.7	3.3	40	120	194	14.9	4.5	42	93
18	72	2	250 µg 16	differ-	20	8.4	1.9	30	160	284	12.5	4.4	42	96
19	57	2	250 µg 13	en	27	11.3	2.3	33	140	221	13.2	4.2	41	96
20	62	3	500 µg 21	peri-	—	7.5	1.6	23	148	600	12.8	4.4	44	98
21	75	5	150 µg 18	ods	—	4.9	1.0	17	167	368	11.8	4.0	39	96
22	76	2	250 µg × 13		—	7.1	1.4	22	154	158	16.2	4.9	47	97

Comments

It is a well-known fact that liver preparations are able to maintain a haematological and neurological remission in pernicious anaemia when they are injected in sufficient dosage. As this result was obtained in the present 45 cases treated with aqueous solutions of crystalline vitamin B_{12} for from 2 to 10 years it must be concluded that this vitamin alone is adequate and that liver extract does not seem to contain any additional unknown haematopoietic factor of importance in this disease.

In 12 of the patients who had previously received other therapy the condition was improved by parenteral therapy with vitamin B_{12}. Most of these patients had received oral preparations with hog pyloric mucosa together with vitamin B_{12} and had become resistant to this therapy. The improvement produced by vitamin B_{12} in the *three* patients who had been on liver treatment does not indicate that vitamin B_{12} is better than liver extract, because these patients had not been controlled to the same extent during

liver therapy as during treatment with vitamin B_{12}

The results obtained here are in accordance with a previously published series of 10 patients with pernicious anaemia (1) who after having received vitamin B_{12} therapy for a couple of years were *given injections of* crude liver extract without any further improvement in the haematological status.

In the present series, vitamin B_{12} had been given in varying doses during different periods This does not reduce *the value of the evidence that vitamin* B_{12} alone is adequate therapy Evaluation of an optimal dosage schedule was not the purpose of the investigation

Summary

Forty three patients with pernicious anaemia were treated with injections of crystalline vitamin B_{12} in aqueous solution After periods of therapy for from 2 to 10 years (average 4 1/2 years) optimal haematological remissions were observed. No neurological manifestations developed during the treatment. It must be concluded that vitamin B_{12} is adequate in the treatment of pernicious anaemia, and that liver extract has no advantages over this drug

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University of Lund, Sweden

A Case of Renal Cortical Necrosis Probably Caused by a Human Equivalent of the Shwartzman Reaction

By

BENGT LINDQVIST, PER ERLANSON and ARNE BRUN

We have found in the literature for the years 1883 to 1961 283 cases of symmetric, renal cortical necrosis verified at autopsy or in a few cases that survived, by renal biopsy or a finding of the typical cortical areas of calcification at a radiographic examination. Of these 283 patients (78 % females and 22 % males) 88 % were under 50 years of age and 17 % under 15. In 58 % the renal damage occurred during pregnancy: in 50 % toxemia of pregnancy and/or ablatio placentae had occurred and in 6 % septic abortion or puerperal sepsis. Severe infections had been present in 28 of all cases, and exogenous intoxication or hemolytic reactions in 7 %. 14 % had various grave surgical or medical basic diseases such as burns, injuries after traffic accidents, pancreatitis, diabetic coma, and addisonian crisis, and in 4 % the renal damage arose without apparent connection with any other disease.

No definite information regarding the pathogenesis of the lesion is provided by this heterogeneous clinical material.

Renal cortical necrosis has been produced in animal experiments, by large doses of pressor substances such as pitressin (9) and adrenalin (27). A case reported by Haft and Prior (16) with cortical necrosis arising after repeated intracardial injections of adrenalin in connection with heart, may be cited as a clinical equivalent of these animal observations.

Because of a similar effect obtained with serotonin in animals, Page and Glendening (26) assumed that in retroplacental hemorrhage cortical necrosis might arise through a thromboplastin bearing material from the placenta being pressed out into the blood stream and producing massive precipitation of fibrin. Large amounts of serotonin would be liberated from the consumed thrombocytes and cause vasoconstriction in the renal cortex. Franklin (11) on the basis of experimental observations, supposed that reflex vascular spasm in the cortex, resulting from uterine expansion, might contribute towards producing this renal damage in obstetric cases.

Cortical necrosis can also be produced with poisons, and with bacterial toxins such as that of the staphylococci (24

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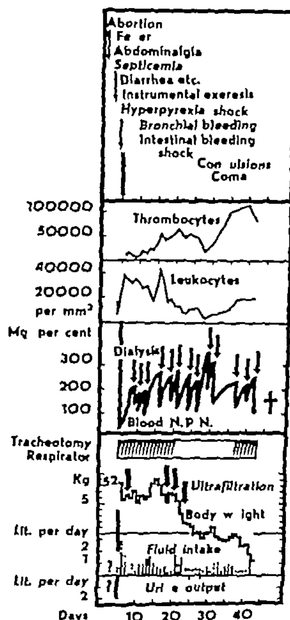


Fig 1 Clinical course. Treatment with the artificial kidney and with respirator

31 a o.) It forms a characteristic feature in the generalized Shwartzman reaction produced by endotoxin (3 10 29 32 33 34 35 36, a. o.)

An intravenous injection of endotoxin in a sublethal dose gives rise to fever shock, leukopenia followed by leukocytosis, thrombocytopenia, petechial hemorrhage, and focal necrosis, particularly in the upper part of the gastro-intestinal tract, and in the spleen and liver The effects of endotoxin is heightened by adrenalin, which, injected intradermally produce necrosis in the infected area in endotoxin-treated animals on the other hand, its action is lowered by corticosteroids.

Two intravenous injections of sublethal doses of endotoxin separated by an interval of about 24 hours produce the generalized Shwartzman phenomenon. This reaction resembles extremely severe endotoxin damage, in connection with which renal cortical necrosis may also arise. An instantaneous decrease in fibrinogen content of the blood takes place, and in the acute stage fibrinous thrombi may be observed in the small vessels in the necrotic tissues. In pregnancy and after treatment with corticosteroids the Shwartzman phenomenon can be produced by a single intravenous injection of endotoxin. Administration of dextran following a sublethal injection of endotoxin will also produce such a reaction.

The occurrence of a corresponding reaction in man has been discussed (5 18, 22 38 a. o.) Cases showing a clinical course resembling a generalized Shwartzman reaction in animals have been described examples of this are shock and anuria following repeated intravenous administration of typhoid vaccine (20, 37) and cases with burns complicated by septicemia due to *Serratia marcescens* or hemolytic streptococci (15 17)

One of the eleven cases of renal cortical necrosis treated at this clinic between 1946 and 1961 (1) ran a course that appeared to resemble the generalized Shwartzman phenomenon¹ (fig 1)

Case report

A 32 year-old woman had had normal deliveries in 1952, 1954 and 1957 She was now (Sept. 1959) in the third month of pregnancy

¹ We are grateful to Professor Rune Grubb, M. D., who drew our attention to the case and aroused our interest in this problem.

On *Day* 1 she tried to induce abortion by pushing a knitting-needle up into the uterus. On *Day* 3 she was experiencing pain in the genital tract, had profuse vaginal discharge, and was hyperpyretic (41.2° C). She was treated with antibiotics (penicillin, streptomycin, and tetracycline) and salicylates and was cooled by washing with alcohol. On *Day* 4 the symptoms from the genital tract were still present, and in addition she now had diarrhea, vomiting, and petechial spots on her face and abdomen. The hemoglobin level was 10.8 g/100 ml. N.P.N. and serum-bilirubin values were normal. The white blood count fell during the day from 13,000 to 7,000 cells/mm³. Hydrocortisone therapy was instituted. A macerated fetus was removed together with the placenta, with little bleeding, by instrumental exeresis and curettage. Culture of secretion from the uterus yielded growth of *Staph. albus* and *E. coli* that were sensitive to most of the antibiotics. Immediately after the operation her systolic blood pressure was 83—90 mm Hg. A few hours later she passed into deep shock, the pulse could not be palpated and the blood pressure could not be measured. Blood transfusions, infusions of dextran and saline solutions, and hydrocortisone failed to produce any improvement. Noradrenalin in larger and larger doses raised the systolic blood pressure to 90—100 mm Hg. *Day* 5. Since the operation on the previous day she had been given more than 4 liters of fluid. The shock persisted, and as she was anuric she was transferred to this clinic. Mentally she was fairly clear. Despite quick infusion of fluid to which 20 mg/l of noradrenalin was added, the blood pressure was only 70/40 mm Hg. The pulse rate was 140 and the respiration rate 48 per minute. She had numerous petechial spots, especially on her nose and fingers, as well as large patches of necrosis on the skin at the sites where the noradrenalin had been infused. Ophthalmoscopic examination revealed retinal edema and spastic arteries. Her abdomen was distended as a result of paralytic ileus. Radiographic examination of the lungs showed mild pulmonary edema and pleural effusion. The hemoglobin levels was 16.8 g/100 ml. She had leukocytosis (39,000/mm³), thrombocytopenia (9,000/mm³), a reduced prothrombin index (60 %) mild uremia (N.P.N. 59 mg/100 ml) and grave acidosis (serum-bicarbonate 8.6 mM). Repeated blood cultures on that day as well as on the following days were negative. Mild hypoxia was present despite hyperventilation. In arterial blood, the pO_2 was 77 mm Hg, the pCO_2 19 mm Hg, the pH 7.34 and the standard bicarbonate 11 mM.

Although noradrenalin was still being administered the blood pressure fell below recordable values, and this drug was therefore withdrawn. Tracheotomy and respirator treatment were necessary because of threatening pulmonary insufficiency and penicillin and kanamycin were administered on suspicion of puerperal sepsis. Vitamin K was given because of the reduced prothrombin index. In order to reduce the acidosis and overhydration, and if possible the degree of intoxication as well, one treatment with the artificial kidney was given the same evening. The treatment corrected the acidosis and reduced the overhydration, but her general condition did not improve. Despite the low blood pressure her mind was still clear. Two hours after the termination of the dialysis treatment, mental confusion and convulsions set in. Infusion of two litres of low-molecular dextran, to which hydrocortisone, papaverine, and pethidine had been added, raised the systolic blood pressure to 80—90 mm Hg and improved her general condition. On *Day* 6 her temperature rose to 41° and fell then gradually to 37—38° C.

During the following days she improved slowly. The thrombocyte count rose from 8,000 to 135,000/mm³. The plasma-fibrinogen level, which was measured for the first time on the 9th day, was 0.17 g/100 ml; 2 days later it was 0.26 g. Fourteen treatments with the artificial kidney were given during the 42 days of the patient's illness. The bleeding tendency especially from the stomach, increased in connection with the heparinizations. A tendency to shock persisted for 2 weeks. The systolic blood pressure remained in the region of 100 mm Hg. Noradrenalin was given on 2 occasions. Admittedly the blood pressure rose in connection with this treatment, but the patient became mentally confused and her general condition deteriorated. After 25 days' anuria the daily urine volume rose to a maximum of 100 ml on the 34th day. Radiographic examination revealed that the kidneys had decreased in size, but

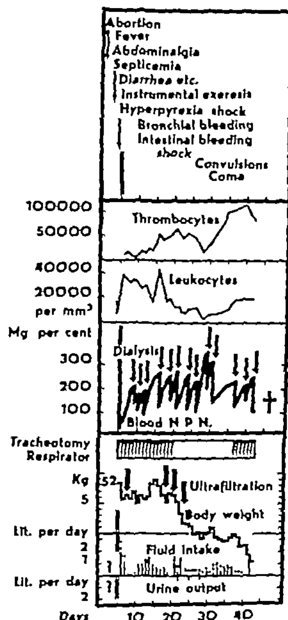


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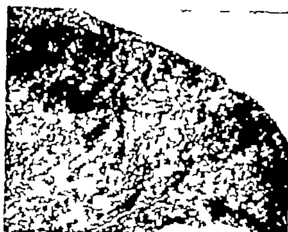


Fig 2. Renal cortical necrosis. Except for the narrow subcapsular zone and the juxta-medullar zone the cortex is necrotic (H & E. x 14)

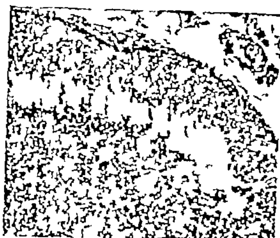


Fig 4 Liver Subcapsular infarcts with peripheral hemorrhagic marginal zone (H. & E. x 34)



Fig 3 Hyaline necrosis of glomeruli and swollen tubular epithelium. (H. & E. x 89)



Fig 5. Adrenal. Infarction t the outer parts of the medulla and f the reticulate zone of the cortex. (H. & E. x 14)

there were no visible areas of calcification. On *Day 36* she suddenly became worse with convulsions, unconsciousness, and irreversible shock. She died on *Day 42*, still unconscious.

Post-mortem findings

The following observ tions were recorded at autopsy Residual changes after induced abortion, with necrotic tissue remains in the uterus bilateral areas of renal cortical necrosis, and nephrosis of the toxic anoxic type multiple areas of necrosis in the liver spleen, and adrenal glands cerebral edema, and extensive necrosis and cell damage in the gray and white matter of the cerebrum and cerebellum hemorrhagic diathesis with hemorrhages in the skin, air passages, and gastro-intestinal tract thrombosis in one of the femoral veins hemosiderosis in the liver and spleen.

The kidneys were smaller than normal and weighed 90 g each. The surface was coarsely granular and yellow to pale-red in color Except for a narrow well-preserved, subcapsular zone and a few radial strands, the cortex, which was only a couple of mm thick,

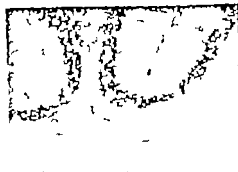
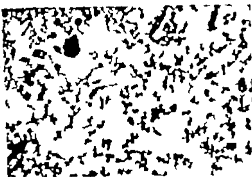



Fig. 6. Fig. 7

Fig. 6 and 7 Cerebellum. Necrosis of the granular layer with destruction of Purkinje's cells and spongy disintegration and vacuolation of the white matter (H. & E. x 34 and x 350 respectively.)

was necrotic, yellowish grey dry and firm almost throughout its entire extent. Histologic examination revealed a fibrinous exudate penetrating the necrotic tissues. In the zone bordering on the normal cortex, there were mild fibrosis and insignificant deposits of round cells. In the outer areas of the necrotic parts thrombi were present inter alia in the arcuate and interlobar arteries, in some places showing incipient organization and recanalization. Existing glomeruli had dilated, blood-filled coils. No calcified areas were visible. The tubules contained a few hyaline and granular cylinders, and the epithelium was in places swollen and showed sparse, finely granular fatty degeneration. Iron pigment was found in the interstitial tissue. The papillae renal pelves, and ureters were normal (figs. 2 and 3).

In the liver there were few recent subcapsular polygonal infarcts, roughly 5 × 5 cm in size, with peripheral hemorrhagic marginal zone (fig. 4). Recent infarcts were also observed in the spleen. In the adrenal glands, infarcts in process of organization with proliferating blood vessels, fibroblasts, and phagocytes containing iron pigment were seen in the outer parts of the medulla and in the reticulate zone of the cortex (fig. 4). The uterine cavity was filled with necrotic, spongy hemorrhagic mass adherent to the walls. A couple of erosion patches were observed in the mucous membrane of the stomach and there were petechial spots in the skin and serous membrane of the heart.

The cerebrum showed signs of increased intracranial pressure. The parenchyma was flabby its cut surfaces were damp and glistening, and its vascular markings were prominent. No thrombus or hemorrhage was seen. In the cerebellum, the granular layer of the cortex was almost entirely necrosed and there was widespread destruction of Purkinje cells. The white matter in the cerebellum showed spongy disintegration and vacuolation similar changes were seen here and there in the white matter of the cerebrum. The myelin sheaths formed a pale network around circular areas of degeneration and showed ball-like swellings. A striking feature was the almost complete absence of glioses and the scanty occurrence of foam cells, a few of which were to be seen around blood vessels (figs. 6 and 7). In some brain nuclei the ganglion cells showed degenerative changes.

Comments

The resemblance to the experimental generalized Shwartzman phenomenon which is produced in rabbits by two intravenous endotoxin injections separated by an interval of 24 hours, is striking in this case. This is particularly noticeable with respect to the initial stages.

1 Primary infection (septicemia? an outflow of endotoxin?) after an attempt to induce abortion. High fever a strongly



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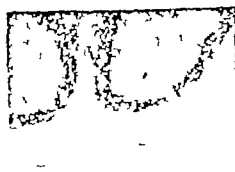


Fig. 6.

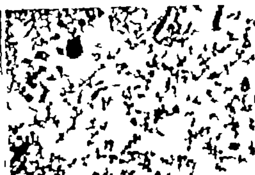


Fig. 7

Fig 6 and 7 Cerebellum. Necrosis of the granular layer with destruction of Purkinje's cells and spongy disintegration and vacuolation of the white matter (H. & E. x 34 and x 350 respectively)

was necrotic, yellowish grey dry and firm almost throughout its entire extent. Histologic examination revealed a fibrinous exudate penetrating the necrotic tissues. In the zone bordering on the normal cortex, there were mild fibrosis and insignificant deposits of round cells. In the outer areas of the necrotic parts thrombi were present inter alia in the arcuate and interlobar arteries, in some places showing incipient organization and recanalization. Existing glomeruli had dilated, blood-filled coils. No calcified areas were visible. The tubules contained few hyaline and granular cylinders, and the epithelium was in places swollen and showed sparse, finely granular, fatty degeneration. Iron pigment was found in the interstitial tissue. The papillae, renal pelves, and ureters were normal (figs. 2 and 3)

In the liver there were a few recent subcapsular polygonal infarcts, roughly 5 × 5 cm in size with peripheral hemorrhagic marginal zone (fig 4) Recent infarcts were also observed in the spleen. In the adrenal glands, infarcts in process of organization with proliferating blood vessels, fibroblasts, and phagocytes containing iron pigment were seen in the outer parts of the medulla and in the reticulate zone of the cortex (fig 4) The uterine cavity was filled with necrotic, spongy hemorrhagic mass adherent to the walls. A couple of erosive patches were observed in the mucous membrane of the stomach and there were petechial spots in the skin and serous membrane of the heart.

The cerebrum showed signs of increased intracranial pressure. The parenchyma was flabby its cut surfaces were damp and glistening, and its vascular markings were prominent. No thrombus or hemorrhage was seen. In the cerebellum, the granular layer of the cortex was almost entirely necrosed and there was widespread destruction of Purkinje's cells. The white matter in the cerebellum showed spongy disintegration and vacuolation similar changes were seen here and there in the white matter of the cerebrum. The myelin sheaths formed a pale network around circular areas of degeneration and showed ball-like swellings. A striking feature was the almost complete absence of glioses and the scanty occurrence of foam cells, few of which were to be seen around blood vessels (figs 6 and 7) In some brain nuclei the ganglion cells showed degenerative changes.

Comments

The resemblance to the experimental generalized Shwartzman phenomenon which is produced in rabbits by two intravenous endotoxin injections separated by an interval of 24 hours, is striking in this case. This is particularly noticeable with respect to the initial stages.

1 Primary infection (septicemia? an outflow of endotoxin?) after an attempt to induce abortion. High fever a strongly



Fig 2. Renal cortical necrosis. Except for the narrow subcapsular zone and the juxta medullar zone the cortex is necrotic (H. & E. x 14)



Fig 4 Liver Subcapsular infarcts with peripheral hemorrhagic marginal zone. (H. & E. x 34.)



Fig 3 Hyaline necrosis of glomeruli and swollen tubular epithelium. (H. & E. x 89)



Fig 5. Adrenal. Infarction at the outer parts of the medulla and of the reticulate zone of the cortex. (H. & E. x 14)

there were no visible areas of calcification On *Day 36* she suddenly became worse with convulsions, unconsciousness, and irreversible shock. She died on *Day 42* still unconscious.

Post-mortem findings

The following observations were recorded at autopsy Residual changes after induced abortion with necrotic tissue remains in the uterus bilateral areas of renal cortical necrosis, and nephrosis of the toxic-anoxic type multiple areas of necrosis in the liver spleen, and adrenal glands cerebral edema, and extensive necrosis and cell damage in the gray and white matter of the cerebrum and cerebellum hemorrhagic diathesis with hemorrhages in the skin, air passages, and gastro-intestinal tract thrombosis in one of the femoral veins hemosiderosis in the liver and spleen.

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affected general condition, diarrhea, vomiting petechial hemorrhage, a relatively low white blood count (7000 cells/mm³)

2. About 24 hours later after instrumental removal of the fetus, deterioration of the general condition with severe prolonged shock that could not be checked by infusions of blood dextran and fluids, and corticosteroids.

3 The patient was pregnant, and was given noradrenalin factors which in animal experiments increase the effect of endotoxin. Areas of necrosis arose in the skin after infusion of noradrenalin

4 The presence of hemorrhagic diathesis thrombocytopenia hypoprothrombinemia, decreased plasma fibrinogen (?) and during the last six weeks of life permanent anuria-oliguria which according to clinical experience may be a condition resulting from cortical necrosis.

5 Autopsy showed areas of necrosis in the renal cortex as well as in the liver spleen adrenal glands and central nervous system. Probably the changes in the kidneys and adrenal glands arose at the onset while the necrosis in the other organs may have developed later

The length of time (42 days) elapsing between the acute onset and death may explain why fibrinous thrombosis in small blood vessels, which is a typical feature of the Shwartzman phenomenon was not observed The clots in process of organization which were observed at the histologic examination may have been a late stage of intravascular fibrinous thrombus formation.

Summary

A case of renal cortical necrosis is presented as a contribution to the discussion on a possible human equivalent of the generalized Shwartzman phenomenon in animals.

A woman of 32 in the third month of pregnancy fell ill two days after an attempt to induce abortion with abdominal pain high fever and septic exanthema. Instrumental abortion performed 24 hours later was followed by severe shock and persisting anuria. She survived for 42 days, during which she received 14 treatments with the artificial kidney

The clinical course showed a striking resemblance to the generalized Shwartzman phenomenon seen in rabbits after two intravenous injections of endotoxin separated by an interval of 24 hours. First there was a primary septic infection and then following an instrumental abortion 24 hours later grave shock, hemorrhagic diathesis, leukopenia, patchy necrosis in the skin after noradrenalin infusions, and persisting anuria. Autopsy showed cortical necrosis and necrotic areas in the liver spleen, adrenal glands, and brain.

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On the Effect of l Triiodothyronine on the Thyroid Gland and its Clinical Application (the Triiodothyronine Suppression Test)

By

Thorkild Friis

Several authors have reported that thyroid hormones suppress the function of the thyroid gland in normal subjects, as measured by the uptake of I[131] in the gland while in thyrotoxic patients no such suppression occurs (5 6 12 20 22, 23 and others) Werner (20 23) is of the opinion that in hyperthyroidism the thyroid gland is not governed in the usual way by the pituitary gland a view which corresponds with the demonstration of a special thyroid-stimulatory substance in the blood in hyperthyroidism (1 9)

Sharer et al. (13) found that in hypophysectomized subjects thyroxine does not further reduce the thyroid uptake of I[131] but that in these individuals thyrotrophic hormone increases the uptake during thyroxine medication. These authors concluded that in normals thyroxine must inhibit the secretion of thyrotrophic hormone, but not its effect upon the thyroid gland.

It has frequently been attempted to utilize the difference between the response of normal and hyperthyroid subjects to thyroid hormone by determining the I[131] uptake in the gland before and during treatment with a suitable dose of l thyroxine or triiodothyronine (5 11 22). This has invariably shown a clear difference between euthyroid and hyperthyroid subjects. Thus, Greer et al. (5) and Werner et al. (22) found the thyroid uptake of I[131] during hormone medication to drop to less than 20 % of the I[131] dosage in normal subjects, while in hyperthyroid subjects it did not drop below 35 % of the dosage.

Others (6, 11) have estimated the reaction of the gland on the basis of the ratio of the I[131] uptake by the thyroid gland before and during hormone medication, which should last 3 weeks with thyroxine and one week with triiodothyronine. This has shown some overlapping be-

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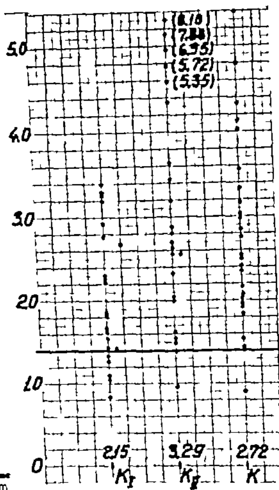


Fig. 1. K values in patients without endocrine disorders. Ordinate: K value (normal > 1.40)

of the residual activity was deducted from the 4-hour determination and 24 % of the residual activity from the 24-hour determination, the effective half-life of the I^{131} remaining in the gland from the 1st determination being taken to be 6 days.

The 4-hour and 24-hour uptakes before the administration of triiodothyronine were then divided by the 4-hour and 24-hour uptakes during triiodothyronine medication, giving two co-efficients K_1 and K_2.

$$K_1 = \frac{\text{4-hour uptake of } I^{131} \text{ before adm. of T}}{\text{4-hour uptake of } I^{131} \text{ during adm. of T}}$$

$$K_2 = \frac{\text{24-hour uptake of } I^{131} \text{ before adm. of T}}{\text{24-hour uptake of } I^{131} \text{ during adm. of T}}$$

The mean values of K_1 and K_2 were calculated = K.

When K was > 1 this was an indication that the glandular function could be suppressed by th triiodothyronine.

As already mentioned, the material comprised total of 127 patients, viz. 26 males and 101 females ranging in age from 21 to 81 years. Of these, 27 had no endocrine disorders, 31 were hyperthyroid, 3 possibly hyperthyroid, 3 hypothyroid, 3 euthyroid with malignant exophthalmos, 4 had undergone thyroidectomy for hyperthyroidism, 25 wer being treated with methyl thiouracil or Carbimazole (Neo-Mercazole) for hyperthyroidism, 19 had diffuse non-toxic goitre

tween euthyroid and hyperthyroid persons 0—1 % of the euthyroid subjects being in the thyrotoxic range and 7—9 % of the thyrotoxic patients in the normal range

A few authors have investigated the findings in surgically treated or drug treated thyrotoxic patients who were euthyroid at the time of the study The results have been divergent. Some found the patients to respond like normal subjects (7 10) while others report that the thyroid function cannot be suppressed by thyroid hormone (15 17 24) Werner (24) for instance, reports that only 17 out of 27 thyroidectomized patients responded in the normal way Vander Laan and Cassidy (17) have reported on hyperthyroid patients on long term anti thyroid medication Administration of thyroxine at the end of one year s treat ment showed that in 5 patients, who later developed recurrences after with drawal of the medication, the I^{131} uptake could not be suppressed while suppressibility was found in 27 patients who did not develop subsequent recurrence In these authors opinion the test is of prog nostic value

Werner et al (21 22) called attention to the interesting fact that euthyroid in dividuals with progressing exophthalmos respond like hyperthyroid patients, while patients with improved exophthalmos give a normal response.

It has moreover been demonstrated by several authors (12 14 22) that the function of non-toxic adenomas cannot be suppressed by thyroid hormone if they are characterized as hot nodules (radioactivity following administration of I^{131} higher in the adenoma than in the surrounding glandular tissue) On the other hand patients with diffuse non toxic goitres generally respond to thyroid hormone by a reduced I^{131} uptake (8) It has been reported that small goitres generally respond mostly as the cases which might respond by a diminution of the goitre to thyroid hormone medica tion In Hashimoto s goitre the glandular function may be suppressed by thyroid hormone even though the gland is being subjected to heavy thyrotrophin stimula tion (19)

On the whole the literature indicates that in hyperthyroid patients the thyroid function cannot be suppressed by thyroid hormone, in contradistinction to the findings in euthyroid subjects except when the latter have non toxic adenomas which often behave like hyperthyroid conditions. In respect to treated hyperthyroid patients who have been rendered euthyroid the views seem to be somewhat conflicting

For the purpose of testing the validity of these reports, and the clinical applica bility of the findings I have carried out a total of 136 tests in the past 18 months on 127 patients with various thyroid disorders.

Methods

The I^{131} uptake by the thyroid gland 4 and 24 hours after oral administration of 10μC I^{131} was determined by the usual technique (3) Furthermore, the PBI^{131} in the serum was determined 24 hours after administration and so were the uptake of I^{131}-labelled triiodothyronine by the red cells (4) and proteinbound iodine in the serum.

After the uptake determinations the patients were given 1-triiodothyronine (Tertroxin, Glaxo) 20 μg four times daily for 8 days. On the 6th day the residual activity in the thyroid gland was determined. On the 7th day another dose of I^{131} (10 μC) was administered, and the 4-hour and 24-hour uptakes were again determined. Out of regard to the effective half-life of the I^{131} remaining in the gland from the previous uptake determination, 13 •

Table I Triiodothyronine suppression test in 31 patients with hyperthyroidism and 3 with questionable hyperthyroidism (nos. 33—36)

No.		Goitre (+ diffuse 0 nodular)	BALR (90—110 %)	PBI (3.0—8.0 μg/100 ml)	T* uptake by red cells (8.0—10.5 %)	PBI^{131} (< 0.3 %/l serum)	^{131}I uptake by the thyroid gland				k (~1.0)
							Before adm. of T		During adm. of T		
							4-hour (15—45 %)	24-hour (30—70 %)	4-hour	24-hour	
1		++	183	>32	12.0	0.67	72.0	92.4	100.0	102.0	0.82
2			163	7.0	11.5	1.00	67.0	68.5	81.5	63.2	0.91
3			161	4.5	9.0	0.35	31.8	47.0	40.0	54.3	0.83
4			156	14.0	17.5	0.78	62.1	80.0	64.0	78.0	1.00
5			151	13.3	17.5	0.41	47.5	80.0	63.0	83.8	0.84
6			150	10.0	16.4	0.36	90.5	100.0	78.5	90.6	1.23
7	Iodine intake	0	150	>32	12.5	0.07	31.8	48.4	29.8	51.0	1.01
8	Iodine intake	00	148	12.7	18.9	0.37	36.0	63.0	47.6	81.0	0.77
9	History of thyroidectomy	00	147	12.4	22.2	0.32	29.2	36.2	24.2	51.0	0.96
10		++	145	3.8	6.4	0.42	71.8	75.0	64.4	62.5	1.16
11		+++	143	11.5	12.7	1.04	77.0	77.0	65.0	80.5	1.07
12		+	141	12.1	15.2	0.47	100.0	98.0	100.0	100.0	0.90
13		+	139	15.0	9.3	0.28	72.0	100.0	57.6	74.6	1.30
14	Iodine intake	+	138	15.1	10.2	<0.01	10.6	15.7	8.8	12.4	1.29
15		+	130	—	8.6	0.10	38.3	31.2	48.0	60.0	0.67
16		+	136	6.9	9.2	0.51	56.2	64.2	49.5	64.0	1.07
17	Iodine intake	0	136	12.3	10.3	0.08	20.5	41.5	28.1	47.5	0.80
18		+	133	—	21.4	0.17	100.0	99.0	92.0	100.0	1.04
19	Exophthalmos	+	133	13.2	15.0	1.14	—	93.5	—	100.5	0.92
20			132	14.8	21.0	0.27	73.5	81.0	118.0	76.5	0.84
21	History of thyroidectomy	0	131	16.2	10.3	0.41	59.3	68.0	76.0	95.0	0.79
22			130	8.7	13.7	0.11	16.4	43.0	32.4	52.9	0.68
23		+	129	7.9	18.3	0.81	95.0	87.0	76.0	64.2	1.5[illegible]
24		0	129	17.9	16.0	0.34	80.0	81.5	99.0	90.0	0.86
25	Exophthalmos	0	126	9.8	10.1	0.80	57.0	84.0	57.0	77.5	1.04
26		+	12[illegible]	8.8	13.4	0.01	17.4	6.6	87.0	73.5	0.15
27	History of thyroidectomy		125	13.0	—	1.84	78.0	72.5	88.5	78.6	0.92
28	Exophthalmos		124	16.6	18.8	0.84	64.0	76.0	59.0	75.0	1.05
29	History of thyroidectomy	0	124	>32	11.8	0.24	47.0	78.0	55.0	90.0	0.88
30		+	117	7.6	11.8	0.72	68.0	90.0	86.0	96.0	0.86
31			107	5.6	9.9	0.70	73.5	83.0	90.5	89.0	0.89
32	Iodine intake	++	—	10.7	16.5	—	21.0	42.7	24.8	41.5	0.94
33		++	119	13.7	18.8	0.02	55.0	57.5	40.0	58.5	1.18
34		+	111	>32	14.0	0.42	93.5	105.0	60.0	82.0	1.32
35		++	111	8.6	16.2	0.03	38.5	53.8	34.4	48.0	1.14
36		++	105	—	8.4	0.02	37.5	67.8	23.2	69.8	1.73
											0.98

Identical patients Nos. 14—15 and 35—36.

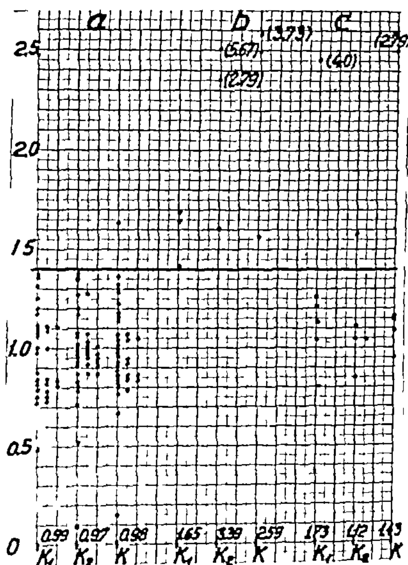


Fig 2 K values in patients w th ( ) hyperthyroidism, (b) hypothyroidism, and (c) euthyroid patients with malignant exophthalmos. Ordinate K alue (normal > 1 40)

— including 2 with Hashimoto s goitre — and lastly 12 had a nodular non toxic goitre

The diagnoses had been made on the basis of the clinical appearances, the B M R., the concentration of protein bound iodine in the serum, and the usual tests with I^{131} (triiodothyronine uptake by the red cells, I^{131} uptake by the thyroid gland, and PBI^{131}) while of course no regard had been paid to the suppression test.

Practically all the patients could tolerate the substance in the dosage used, even the hyperthyroid patients. Presumably there is little risk of appreciable exacerbation in these cases, cf the fact that thyrotoxic crisis seldom develops when thyroid hormone is administered for short periods and in moderate doses (18) However the test had to be interrupted in 2 cases (a hypothyroid and a hyperthyroid patient) because of tachycardia.

Results

1 Patients without endocrine disorders

The result of the suppression test in these 27 patients is given in fig 1 Eight out of 19 patients showed a slightly ele-

2. PATIENTS WITH HYPER- AND HYPO-THYROIDISM

This group comprised 31 patients with definite hyperthyroidism 3 with questionable hyperthyroidism, and 5 with hypothyroidism. The results are set out in tables I and II as well as fig 2. One patient with hyperthyroidism and one with questionable hyperthyroidism were subjected to 2 tests. Among the patients of this group there were 16 with diffuse goitre and 8 with adenoma. The type and size of the goitre did not appear to influence the extent of the I^{131} uptake. Three had exophthalmos, 4 had a history of thyroidectomy for hyperthyroidism, and 5 had received iodine in some form or other during the past month, which reduces the I^{131} uptake and PBI^{131} and increases the PBI

Among the hyperthyroid patients the parameters were as follows The B.M.R. was elevated in 31 out of 35 PBI was elevated in 26 out of 33 and PBI^{131} in 22 out of 35. The T uptake by the red cells was increased in 24 out of 35 the 4-hour uptake of I^{131} by the thyroid gland in 24 out of 35 and the 24-hour uptake in 20 out of 36. In other words, all the tests, except the B.M.R., showed a positive result in about three-quarters of all cases.

As regards the suppression test Among the K_1 values one exceeded the 1 40 limit (questionable thyrotoxicosis) Among the K_4 values none and among the K values one exceeded 1 40 (questionable thyrotoxicosis) Since the smallest number of values was below 1 40 for K and K_4 in the normal group, either K_4 or K must be best suited for distinguishing between hyperthyroidism and euthyroidism, only one determination having overlapped. It may be seen, moreover (fig 2) that unlike the findings in the normal groups (fig 1) the K_1 K_4, and K values were practically identical. In the case of the 24-hour uptakes during triiodothyronine medication it will be seen that all values exceeded 50 % (except in a questionably thyrotoxic patient) with the exception of those in the 5 patients who had received iodine. A comparison with the 24-hour uptakes during triiodothyronine medication in the normal groups, in which only one value exceeded 50 % (60.5 %) shows that in this respect too there is a clear distinction. In the 4-hour uptakes there was more overlapping

Fifteen determinations showed that the glandular function could be slightly suppressed 20 that the gland increased its I^{131} uptake, in several cases even considerably This phenomenon is unexplained. Strange to say the 8 patients with nodular goitre showed either an increased or an unchanged I^{131} uptake during triiodothyronine medication Incidentally the size of the goitre did not influence the result of the suppression test. In the 3 cases with exophthalmos the thyroid function could also not be suppressed

In the suppression test the 5 myxoedematous patients behaved like the normal subjects (table II and fig 2) In these cases, however the appraisal is complicated by the fact that the uptakes were low the recorded radioactivity in the gland being low and the uncertainty thus greater

Among the 5 patients who had received iodine during the preceding month the usual I^{131} tests gave misleading results (except for the T uptake by the red cells which fell owing to the iodine medication which acts as treatment) These 5 patients also showed low K values.

The relationship between the 4-hour uptake before administration of triiodothyronine and K_1, between the 24-hour

Table II Triiodothyronine suppression test in 3 hypothyroid patients

No.	B.M.R (90—110 %)	PBI (3 0—8.0 µg/100 ml)	T uptake by red cells (6.0—10.5 %)	PBI (<0.3 %/l serum)	I^{131} uptake by the thyroid gland: Before adm. of T, 4-hour (15—45 %)	Before adm. of T, 24-hour (30—70 %)	During adm of T, 4-hour	During adm of T, 24-hour	K (>1 40)
1	83	1.2	5.2	0.10	10.2	11.9	5.7	2.1	3.73
2	73	2.2	6.9	0.22	10.8	9.5	7.6	5.6	1.56
3	88	2.8	10.5	0.26	17 6	27.0	10.1	9.7	2.49
									2.59

Table III Triiodothyronine suppression test in 3 euthyroid patients with malignant exophthalmos

No.	B.M.R. (90—110)	PBI (3 0—8.0 µg/100 ml)	T uptake by red cells (6.0—10.5 %)	PBI (<0.3%/l serum)	I^{131} uptake by the thyroid gland: Before adm. of T, 4-hour (15—45 %)	Before adm. of T, 24-hour (30—70 %)	During adm of T, 4-hour	During adm of T, 24-hour	K (>1 40)
1	95	5.8	4.9	0.36	42.5	52.4	37 4	31 4	1 09
2	92	4.2	8.7	0.37	62.0	63.0	60.5	74.0	0.93
3	108	—	10.6	0 17	53.6	66.2	13.4	43.6	2.79
4	83	4 6	—	0.37	33.4	51 0	27.6	45.9	1 16
5	95	—	6.9	0.1	29.0	52.1	23.1	50.3	1 15
									1 43

Identical patients Nos. 2—3 and 4—5.

vated B.M.R 2 out of 17 had elevated PBI 4 out of 27 had an increased T_3 uptake by the red cells, all had normal PBI^{131} 3 had an increased 4-hour uptake and one an increased 24-hour uptake of I^{131} by the thyroid gland while one had a reduced 4-hour and 24-hour uptake. All these alterations were estimated as being of no definite clinical significance.

In 25 out of the 27 patients the 24-hour uptake of I^{131} could be suppressed by triiodothyronine to such an extent that $K_2 > 1.40$ As far as the 4-hour uptake was concerned 5 were below this value. Only one had a mean value K of < 1 40 A comparison of K_1 with K_2 shows that on the whole K_2 was higher than K_1 which means that the 24-hour uptakes were more easily suppressed There was no relationship between K values and uptake values before the administration of triiodothyronine, and the suppression test was also not influenced by sex and age. Concerning the 24-hour uptakes during administration of triiodothyronine only one showed a value exceeding 30 % i. e 60 5 %

2. PATIENTS WITH HYPER- AND HYPO-THYROIDISM

This group comprised 31 patients with definitive hyperthyroidism, 3 with questionable hyperthyroidism, and 3 with hypothyroidism. The results are set out in tables I and II as well as fig 2 One patient with hyperthyroidism and one with questionable hyperthyroidism were subjected to 2 tests. Among the patients of this group there were 16 with diffuse goitre and 8 with adenoma. The type and size of the goitre did not appear to influence the extent of the I^{131} uptake Three had exophthalmos, 4 had a history of thyroidectomy for hyperthyroidism, and 5 had received iodine in some form or other during the past month which reduces the I^{131} uptake and PBI^{131} and increases the PBI.

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As regards the suppression test Among the K values one exceeded the 1 40 limit (questionable thyrotoxicosis) Among the K values none and among the K values one exceeded 1 40 (questionable thyrotoxicosis) Since the smallest number of values was below 1 40 for K and K_4 in the normal group either K or K must be best suited for distinguishing between hyperthyroidism and euthyroidism only one determination having overlapped. It may be seen, moreover (fig. 2) that unlike the findings in the normal groups (fig 1) the K_1, K_4, and K values were practically identical. In the case of the 24-hour uptakes during triiodothyronine medication it will be seen that all values exceeded 50 % (except in a questionably thyrotoxic patient) with the exception of those in the 5 patients who had received iodine. A comparison with the 24-hour uptakes during triiodothyronine medication in the normal groups, in which only one value exceeded 30 % (60.5 %) shows that in this respect too there is a clear distinction. In the 4-hour uptakes there was more overlapping

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In the suppression test the 3 myxoedematous patients behaved like the normal subjects (table II and fig 2) In these cases, however the appraisal is complicated by the fact that the uptakes were low the recorded radioactivity in the gland being low and the uncertainty thus greater

Among the 5 patients who had received iodine during the preceding month the usual I^{131} tests gave misleading results (except for the T uptake by the red cells which fell owing to the iodine medication which acts as treatment) These 5 patients also showed low K values.

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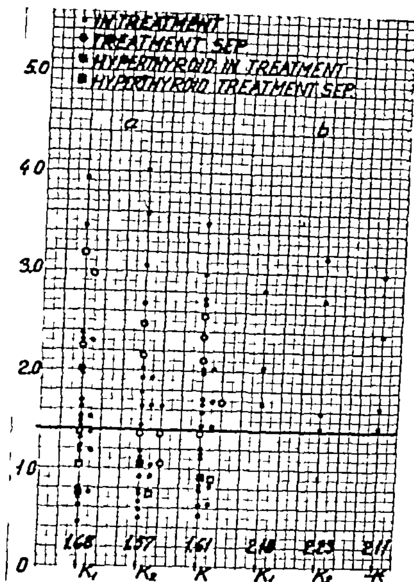


Fig 3 K values in hyperthyroid patients treated with ( ) antithyroid medication and (b) thyroidectomy
Ordinate K value (normal > 1 40)

uptake before the administration of triiodothyronine and K_2 and between PBI[131] and K were analysed without showing any definite relationship

Malignant exophthalmos

Three euthyroid patients with malignant exophthalmos were included in the study All three had undergone thyroidectomy for hyperthyroidism and the progressive exophthalmos had developed after the operation. In addition to severe exophthalmos they had pareses of ocular muscles and chemosis. The patients were not being treated at the time of the test. The results are shown in table III and fig 2

Case 1 was just developing her exophthalmos. The ordinary thyroid tests were normal apart from PBI[131] which was slightly elevated as a sign of a rapid turnover of I[131] in the gland remnant. It will be seen that the glandular function could not be suppressed by triiodothyro-

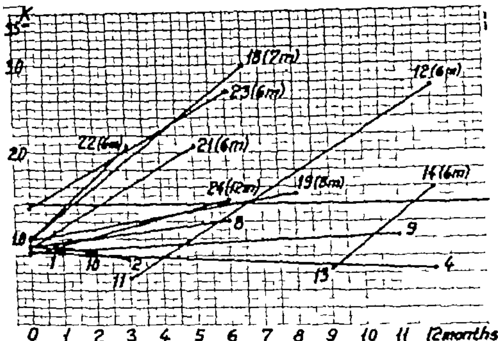


Fig. 4 K values in hyperthyroid patients on antithyroid medication. Abscissa: Months after commencement of treatment. Ordinate: K value. The figures refer to the numbers in table IV. Figures in brackets: Follow-up period after discontinuation of medication.

nine. The other 2 patients were tested twice at an interval of one year. In the meantime they were treated with prednisone. Both showed the same results as case 1, viz. that the glandular function was not suppressible by triiodothyronine in the former test.

Case 2 improved on prednisone, and her glandular function could be suppressed at the latter test. The third patient did not improve, and in her case the response to the suppression test was unchanged. In other words, the thyroid gland in these cases reacted to triiodothyronine in the same way as in hyperthyroid patients.

3. Treated hyperthyroidism

A total of 29 treated hyperthyroid patients were tested, viz. 25 treated by antithyroid drugs and 4 by thyroidectomy for hyperthyroidism. The results may be read from tables IV and V as well as figs. 3, 4 and 5.

Drug-treated

Thirty tests were performed on the 25 drug-treated patients. Ten had previously been tested before the institution of therapy (fig. 4). All 10 had been thyrotoxic at the time, having K values below 1.40 (cases 4, 8, 9, 10, 18, 19, 21, 22, 23, and 24). Five (cases 3, 8, 16, 21, 22) had a history of thyroidectomy for hyperthyroidism and had relapsed.

Two patients (cases 1 and 17) were thyrotoxic at the time of the testing. Both had K values lower than 1.40. All the others were assessed as being clinically euthyroid except one who was hypo-

Table II Triiodothyronine suppression test in 25 patients on antithyroid medication

No.	Treatment	Clinical data	Goitre (+: diffuse; 0 nodular)	B.M.R. (90–110 %)	T uptake by red cells (6.0–10.5 %)
1	MTU for 3 weeks	Hyperthyr	+	121	13.7
2	MTU for 3 months	Euthyr	+	106	8.4
3	Neom for 6 months discont. ⟶ recurr	Exophth. Hist. of thyroidect.	0	120	10.7
4	MTU for 12 months discont ⟶ recurr			92	4.3
5	MTU for 3 years discont., no recurr		0	130	9.6
6	MTU for 12 months discont no recurr	Exophth.		88	–
7	MTU discont for 12 months	Exophth.		90	10.3
8	MTU for 6 months	Hist. of thyroidect.		116	10.8
9	MTU for 11 months	Exophth.		102	7.1
10	MTU for 2 months		0	90	4.2
11	MTU for 3 months			94	5.9
12	MTU for 12 months discont no recurr			107	3.2
13	MTU for 9 months		++	90	5.6
14	MTU for 12 months discont., no recurr		++	116	9.5
15	MTU discont for 12 months		+	92	8.1
16	MTU for 6 months discont. ⟶ recurr	Hist. of thyroidect.	++	102	6.6
17	MTU discont. for 6 months	Hyperthyr		136	18.1
18	MTU for 6 months discont., no recurr		0	110	9.6
19	Neom for 8 months discont., no recurr			110	6.0
20	MTU for 18 months discont., no recurr		++	108	6.7
21	MTU for 5 months discont., no recurr	Hist. of thyroidect.		104	7.4
22	MTU for 3 months discont., n recurr	Hist. of thyroidect.	0	99	7.4
23	MTU for 6 months discont. n recurr		+	96	9.6
24	MTU for 6 months discont. no recurr			93	7.3
25	MTU for 18 months discont., no recurr		0	88	4.8
26	MTU for 6 months		+	89	6.7
27	MTU for 2 months			140	11.5

PBI^{131} (<0.3 %/l serum)	I^{131} uptake by the thyroid gland				K (>1.40)
	Before adm. of T		During adm. of T		
	4-hour (15—45 %)	24-hour (30—70 %)	4-hour	24-hour	
0.01	39.0	21.6	54.0	20.5	0.91
0.01	43.0	47.5	—	60.0	0.79
0.49	42.5	72.0	35.6	65.0	1.15
0.10	37.0	62.0	61.0	85.7	0.63
0.22	11.8	4.4	7.9	5.9	1.12
0.57	80.5	85.5	68.0	82.0	1.11
0.15	33.4	58.8	15.9	23.8	2.35
0.28	68.0	78.2	42.2	100.0	1.20
0.12	56.1	75.0	72.6	82.8	0.84
0.10	24.1	20.6	33.5	22.2	0.83
—	34.5	48.5	75.5	87.5	0.53
0.08	41.5	40.5	29.3	10.0	2.73
0.06	22.6	26.2	28.2	32.5	0.6[illegible]
0.60	82.0	87.0	41.0	53.0	1.[illegible]8
0.07	46.2	65.2	33.4	48.0	1.37
0.15	42.5	66.3	25.2	40.1	1.67
1.10	70.6	61.0	68.0	79.0	0.90
0.11	49.6	76.0	20.8	21.2	2.98
0.21	56.5	78.4	37.1	57.2	1.45
0.06	31.6	43.6	24.2	24.6	1.99
0.08	31.0	17.8	13.4	10.0	2.05
0.08	41.2	52.5	19.1	27.1	2.05
2.25	100.0	29.0	30.3	29.0	2.69
0.29	49.7	44.8	41.0	26.9	1.43
1.07	79.2	73.3	20.2	24.0	3.49
0.65	67.0	66.8	34.2	48.0	1.71
0.64	34.8	59.0	23.2	29.2	1.70

Table II. Triiodothyronine suppression test in 25 patients on antithyroid medication

No.	Treatment	Clinical data	Goitre (+ diffuse; 0: nodular)	B.M.R. (90–110%)	T uptake by red cells (6.0–10.5%)
1	MTU for 3 weeks	Hyperthyr	+	121	13.7
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8	MTU for 6 months	Hist. of thyroidect.		116	10.8
9	MTU for 11 months	Exophth.		102	7.1
10	MTU for 2 months		0	90	4.[illegible]
11	MTU for 3 months			94	5.9
12	MTU for 12 months discont., no recurr			107	5.2
13	MTU for 9 months		++	90	5.6
14	MTU for 12 months discont., no recurr		++	116	9.5
15	MTU discont. for 12 months		+	92	8.1
16	MTU for 6 months discont. → recurr	Hist. of thyroidect.	++	102	6.6
17	MTU discont. for 6 months	Hyperthyr		136	18.1
18	MTU for 6 months discont., no recurr		0	110	9.6
19	Neom. for 8 months discont., no recurr			110	6.0
20	MTU for 18 months discont. no recurr		++	108	6.7
21	MTU for 5 months discont., no recurr	Hist. of thyroidect.		104	7.4
22	MTU for 3 months discont., no recurr	Hist. of thyroidect.	0	99	7.4
23	MTU for 6 months discont no recurr		+	96	9.6
24	MTU for 6 months discont., no recurr			93	7.3
25	MTU for 18 months discont., no recurr		0	88	4.8
26	MTU for 6 months		+	89	6.7
27	MTU for 2 months			140	11.5

PBI^{131} (<0.3 %/l serum)	I^{131} uptake by the thyroid gland				K (>1.40)
	Before adm. of T		During adm. of T		
	4-hour (15—45 %)	24-hour (30—70 %)	4-hour	24-hour	
0.01	57.7	69.6	19.4	52.3	2.57
1.52	66.0	80.0	32.9	58.0	1.69
0.49	93.5	91.0	29.4	86.5	2.12

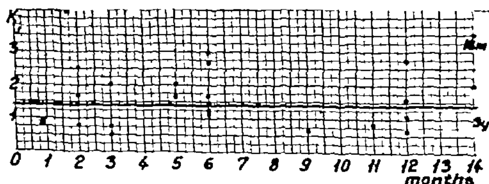


Fig. 5. Relationship between K value and duration of treatment by antithyroid drugs. Abscissa: Duration of treatment in months. Ordinate: K value.

thyrotoxic (cases 1 and 17). The others were euthyroid and had been treated for periods of from 2 months to 3 years (table IV). In one the treatment had been discontinued at the time of the test (case 15). Among these 10 euthyroid patients on antithyroid medication having a K value of less than 1.40 the treatment was discontinued in 4 (cases 3, 4, 5 and 6) and two (cases 3 and 4) developed a recurrence of thyrotoxicosis in 2—3 months. In the remaining six (cases 2, 8, 9, 10, 11 and 13) the treatment was continued. Two were tested a few months later (cases 12 and 14) and now the K value was found to be > 1.40. The treatment was then stopped, and there have been no recurrences.

Among the remaining patients 14 K values were found to be > 1.40 (cases 16, 18—30). In three (cases 28, 29 and 30) the treatment had been withdrawn at the time of the test. In 2 (cases 29 and 30) the medication had not been withdrawn until a few days previously, so their thyroid glands were still under a heavy thyrotrophin stimulation (16). PBI^{131} was elevated in these 2 patients. The fact that their thyroid function could be suppressed by triiodothyronine, unlike the hyperthyroid patients, indicates that stimulation of the thyroid gland in hyper-

Table II Cont

No.	Treatment	Clinical data	Goitre (+ diffuse; 0 nodular)	B.M.R. (90—110)	T uptake by red cells (6.0—10.5 %)
28	MTU for 2 months discont 2 weeks previously			112	9.4
29	MTU for 12 months discont. days previously			134	9.1
30	MTU for 3 years discont. 2 days previously	Myxoedema		69	7.6

Identical Nos. 1—2 6—7 11—12 13—14 and 16—17

Table I Triiodothyronine suppression test in 4 patients thyroidectomized for hyperthyroidism

No.	B.M.R. (90—110 %)	PBI (3.0—8.0 μg/100 ml)	T uptake by red cells (6.0—10.5 %)	PBI¹³¹ (< 0.3% l serum)	I uptake by the thyroid gland				K (>1.40)
					Before adm. of T		During adm. of T		
					4-hour (15—45 %)	24-hour (30—70 %)	4-hour	24-hour	
1	120	5.6	6.3	0.19	22.0	48.9	10.8	17.9	2.38
2	116	7.4	8.1	0.01	49.5	74.0	17.5	23.4	.99
3	102	7.5	8.3	0.33	41.1	63.5	24.9	40.0	1.63
4	114	6.4	7.6	0.70	32.4	59.8	—	41.9	1.43
									2.11

thyroid (case 30) The parameters were as follows B.M.R. elevated in 9 and reduced in 4 PBI was only determined in a few cases of this group the T_3 uptake by the red cells was increased in 5 and reduced in 6 PBI¹³¹ was elevated in 9 (in 2 the antithyroid treatment had been withdrawn 2 days previously which entails an elevated PBI¹³¹ owing to an increased turnover in the gland due to a pronounced thyrotrophin stimulation one had previously undergone thyroidectomy and one had exophthalmos, factors which may give rise to an elevated PBI¹³¹ in euthyroid subjects (3)) In respect to the I¹³¹ uptake determinations table IV shows that the 4-hour uptake was increased in 15 reduced in 1 and that the 24-hour uptake was increased in 10 and reduced in 6 Thus, in drug treated hyperthyroid patients the I¹³¹ uptake and PBI¹³¹ are extremely variable a finding which corresponds with those reported previously (3) The dosage of methyl thiouracil was usually 100 mg daily except in case 1 who was on a higher dosage and in cases 7 15 17 28 29 and 30 in whom the medication had been withdrawn before the test (cf table IV) The daily dosage of Neo-Mercazole was 10 mg

As regards the K values 13 were < 1.40 Among these patients 2 were

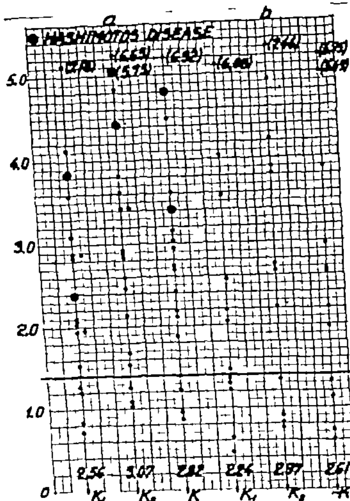


Fig. 6. K values in patients with (a) non-toxic diffuse and (b) non-toxic nodular goitre. Ordinate: K value (normal > 1.40)

have an I^{131} uptake exceeding 50 % during the administration of triiodothyronine unless they had been receiving iodine. This did not apply in the present group, where 3 with K values lower than 1.40 had a 24-hour I^{131} uptake of less than 30 % during the administration of triiodothyronine (cases 1, 5 and 10).

Surgically treated

This group comprised 4 patients thyroidectomized for hyperthyroidism 1—3 years previously. All gave a normal response to the suppression test (fig. 3). PBI^{131} was elevated in 2, a phenomenon well-known in thyroidectomized patients.

4. Non-toxic goitre

Nineteen patients had diffuse and 12 nodular goitres. Two of the former had Hashimoto's goitre (positive biopsy and precipitin reaction to thyroglobulin in the serum). Out of the 19 patients with diffuse goitre (table VI, fig. 6) 8 had an

Table VI Triiodothyronine suppression test in 19 patients with diffuse non-toxic goitre

No.	B.M.R. (90—110 %)	PBI (3.0—8.0 μg/100 ml)	T uptake by red cells (6.0—10.5 %)	PBI¹ (<0.3 %/l serum)	I^{131} uptake by the thyroid gland: Before adm. of T, 4-hour (15—45%)	Before adm. of T, 24-hour (30—70%)	During adm. of T, 4-hour	During adm. of T, 24-hour	K (>1 40)	
1	118	14.3	6.1	0 01	?8.8	57.9	13.8	15.1	2.95	
2	115	5.8	9.1	0.29	38.4	61.5	9.3	12.7	4 49	
3	114	8.4	9.3	0.06	24 6	44.3	16.0	15.4	2.19	Hist. of thyroidect. Hist. of hyperthyr
4	110	5.0	8.8	0 02	51 0	64.5	16.4	22.0	3.02	
5	109	7.6	10.5	0.02	46.3	60.2	22.2	28.0	2 12	
6	108	5.4	5.6	<0.01	49.0	70.5	17.3	27 1	2.72	
7	107	5.7	8.3	0.03	97 0	100.0	47.0	59 1	1.88	
8	106	3 7	7.6	0.22	64 0	74 0	8.9	11 1	6.92	
9	101	8.1	[illegible] 0	0.03	47.5	65.3	16.4	26.4	2.68	
10	101	—	9 6	0.30	40.7	57 0	34 0	45.0	1.23	Hist. of thyroidect.
11	100	11.5	8.5	0 19	32.0	54.8	16.4	19 0	[illegible].42	Hist. of thyroidect. Hist. of hyperthyr.
12	9	9 4	8.7	0.10	29.0	59 0	41 0	56.5	0.88	
13	94	7 6	7.2	0.14	20.6	43.5	16.3	28.4	1 40	
14	93	4.9	7 9	<0.01	62.2	88.3	31.6	25.8	2.70	
15	93	5.9	8.9	<0.01	60.2	90.9	21 6	25.8	3.15	
16	91	5.5	7.8	0.03	35.0	59.0	9 8	16.3	3.60	
17	82	5.6	6.9	0.05	12.2	19.8	14.6	18.1	0.97	
18	110	4 4	5.9	0.62	31.[illegible]	50.5	8.1	8.8	4.79	Hashimoto's goitre
19	84	7 1	6.6	0.89	18.4	34.0	7 7	3.1	3.40	Hashimoto's goitre
									2.82	

thyroid patients is qualitatively of a different nature Among the 11 euthyroid patients on antithyroid medication at the time of the test who had K values > 1 40 i.e. in the normal range the treatment was withdrawn in 9 (cases 16 18—25) and one (case 17) had a recurrence in 6 months.

It was investigated whether a relationship existed between the duration of treatment and the K value. This does not seem to be so (fig 5) There was also no relationship between the K value and the I^{131} uptake prior to the administration of triiodothyronine. As already mentioned all the hyperthyroid patients proved to

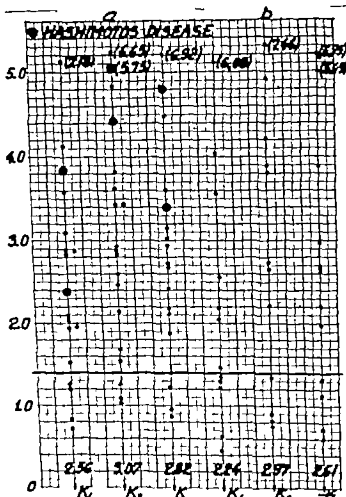


Fig. 6. K values in patients with ( ) non-toxic diffuse and (b) non-toxic nodular goitre. Ordinate K value (normal > 1 40)

have an I[131] uptake exceeding 50 % during the administration of triiodothyronine unless they had been receiving iodine. This did not pply in the present group, where 3 with K values lower than 1 40 had 24-hour I[131] uptake of less than 50 % during the administration of triiodothyronine (cases 1 5 and 10)

Surgically treated

This group comprised 4 patients thyroidectomized for hyperthyroidism 1—3 years previously All gave a normal response to the suppression test (fig. 3) PBI[131] was elevated in 2, a phenomenon well-known in thyroidectomized patients.

4 Non-toxic goitre

Nineteen patients had diffuse and 12 nodular goitres. Two of the former had Hashimoto s goitre (positive biopsy and precipatin reaction to thyroglobulin in the serum) Out of the 19 patients with diffuse goitre (table VI fig 6) 8 had an

Table III Triiodothyronine suppression test in 12 patients with non-toxic adenoma

No.	B.M.R. (90—110 %)	PBI (3.0—8.0 μg/100 ml)	T uptake by red cells (6.0—10.5 %)	PBI (<0.3 μg/l serum)	I^{131} uptake by the thyroid gland: Before adm. of T, 4-hour (15—45 %)	Before adm. of T, 24-hour (30—70 %)	During adm. of T, 4-hour	During adm. of T, 24-hour	K (>1 40)	
1	139	7 4	9.3	—	41 0	55.0	16.0	20.7	2.62	
2	125	2 8	7 7	1.36	65.0	73.0	50.0	53.8	1.33	
3	123	4.2	5.0	0.29	38.4	53.0	9.5	7 1	5.75	Hist. of thyroidect.
4	109	12.2	8.8	0.04	26.9	52.0	18.3	13.7	2.64	Hist. of thyroidect.
5	106	9.5	10.3	0 42	22.0	35.0	10 7	9 0	2.97	Iodine intake
6	102	4 6	6.9	0.08	38.4	60 0	28.4	66.5	1 13	
7	97	8.8	9 0	0.21	54 0	74.5	8.9	15.2	5.49	
8	99	8.4	5.3	0.04	17 4	27 4	17 4	10.0	1.98	
9	92	7.2	6.1	0.16	20 0	44 0	43.6	37.8	0.61	
10	85	6.7	11 1	0.10	42.7	69 0	19 4	31.2	2.21	
11	118	5.8	8.1	0 04	3_4	53.3	51 0	66.5	0.73	
12	100	4.2	6 0	0.06	48.0	78.7	13.5	18.7	3.88	
									_61	

increased uptake of I^{131} in the gland a finding which has been reported previously (3) Three had a slightly elevated B M R. 5 an elevated PBI and no elevation of the T_3 uptake by the red cells. All had a normal PBI^{131} with the exception of the two having Hashimoto's goitre in whom it was increased — another well known phenomenon Three of the total group showed no suppression on triiodothyronine (K < 1 40) a finding which is so far unexplained Three of the patients had a history of thyroidectomy (cases 3 10 and 11) in two cases because of hyperthyroidism In the latter two the K value was > 1 40

Among the 12 patients with nodular goitres the B M.R. was elevated in 4 the PBI elevated in 4 the T_3 uptake by the red cells increased in 1 the PBI^{131} elevated in 2 and the I^{131} uptake in 3 In 4 the glandular function could not be suppressed by triiodothyronine. Two had a history of thyroidectomy for non-toxic goitres (cases 2 and 3)

As in the other groups, there was no relationship between the extent of the uptake and the K values, and there was also no relationship between the size of the goitre and the K values.

Discussion and conclusion

In the present series there was a clear difference between the normal group and the thyrotoxic group only one normal subject having a K value < 1 40 (4 1 %)

and only one, with questionable thyrotoxicosis, a K value > 1.40 (2.9 %). This is in keeping with previous findings (5, 6, 11 and 22). In this respect, therefore, the test is superior to other thyroid tests and is consequently a valuable supplement for clinical use. A snag about this test is that the patients have to be treated with triiodothyronine for 8 days and that they must have 2 radioactive tests, although the dosage is low.

Among drug-treated thyrotoxic patients, in whom the ordinary tests give varying results, the suppression test appears to be of importance. If thyroid function can be suppressed during treatment with triiodothyronine, the patient is usually not thyrotoxic. However in several treated patients it proved impossible to suppress the thyroid function (K < 1.40) although the patients were not thyrotoxic. Whether the test may acquire prognostic value is still open to question, as the material does not permit of any conclusion. Two out of four patients, whose thyroid function was not suppressible, developed a recurrence of thyrotoxicosis after discontinuation of treatment as did 1 out of 9 patients who could be suppressed. Continued investigations into this item are in progress.

It is interesting to note that patients with malignant exophthalmos respond like thyrotoxic patients, even when they are euthyroid. This indicates that their thyroid glands are under abnormal stimulation, although the patients are euthyroid.

The patients with a history of thyroidectomy for hyperthyroidism who were now euthyroid comprised in addition to 4 in the thyroidectomized groups, 5 in the drug-treated group, 3 in the group having malignant exophthalmos, and 2 in the group having diffuse non-toxic goitre. In all cases the thyroidectomy had been performed more than a year previously. All gave a normal response to the suppression test except 2 in the drug-treated group and the 3 with malignant exophthalmos. In one of the latter however the test became normal after the exophthalmos had subsided. As already mentioned there seems to be some difference of opinion regarding the suppression test in thyroidectomized patients. It would be of interest to ascertain how long after the operation thyroidectomized hyperthyroid patients with normal response to the suppression test "switch over" from an abnormal to a normal K value.

A disadvantage of the suppression test is that 3 out of 19 patients with diffuse goitre and 4 out of 12 with nodular non toxic goitre did not respond by a reduced uptake during the administration of triiodothyronine.

When compared with the reports of previous authors, the present findings show conformity in several respects. There is a clear distinction between hyperthyroid and normal subjects (5, 6, 11, 22); drug treated thyrotoxic patients can often not be suppressed by triiodothyronine, even though they are euthyroid (15, 17, 24); patients with malignant exophthalmos cannot be suppressed (21, 22); in several instances non-toxic adenomas do not reduce the I[131] uptake during administration of triiodothyronine (12, 14, 22); and patients who have undergone thyroidectomy for hyperthyroidism respond like normals, when a certain period has elapsed after the operation (10).

It is evident from the present material that it is not justifiable, as done by certain American authors (5, 22), to use values of < 20 % 24-hour uptake during administration of triiodothyronine as a

criterion of euthyroidism This value should rather be 30 %. However, the K value is preferable as intake of iodine, which is often difficult to trace, will cause the 24-hour uptake during administration of triiodothyronine to be lower than 30 % although the K value is < 1 40 Moreover 3 patients of the drug treated group and 1 patient of the group having diffuse, non-toxic goitre also showed a low 24-hour uptake during administration of triiodothyronine, the K value being lower than 1 40

All considered it may be said that if a patient's thyroid function *can be suppressed* by triiodothyronine (K > 1 40) *hyperthyroidism is extremely unlikely* while the reverse viz. that the patient has hyperthyroidism if the thyroid function *cannot be suppressed* (K < 1 40) does not apply as several patients with non toxic goitres and several drug treated patients who have had hyperthyroidism do not show a reduced thyroid function during administration of triiodothyronine.

Summary

The triiodothyronine suppression test was performed on 127 patients (27 without endocrine disorders, 31 hyperthyroid 3 questionably hyperthyroid 3 hypothyroid 3 euthyroid with malignant exophthalmos, 4 thyroidectomized for hyperthyroidism 25 on antithyroid medication, 19 with diffuse non toxic goitre and 12 with nodular non toxic goitre) The result is expressed in the K value The relationship between the I^{131} uptake before and during the administration of triiodothyronine 20 μg four times daily for 8 days. This value is > 1 40 in euthyroid subjects. There was a clear distinction between the normal group and the hyperthyroid group only 4 1 of the subjects in the normal group and 2.9 % of those in the hyperthyroid group overlapping

Among the drug treated hyperthyroid patients, 10 who had been rendered euthyroid failed to respond to triiodothyronine by a suppression of thyroid function. In 4 the treatment was discontinued after which 2 developed recurrence. In the remaining patients the thyroid function could be suppressed The treatment was withdrawn in 9 one of whom developed a recurrence.

The 3 patients with malignant exophthalmos behaved like the hyperthyroid patients the 4 thyroidectomized patients like normal subjects. Out of 19 with diffuse, non-toxic goitres and 12 with nodular non toxic goitres 3 and 4 respectively did not respond to triiodothyronine by suppression This reduces the value of the suppression test as a diagnostic aid in hyperthyroidism with goitre.

Acknowledgement

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Catabolism and Distribution of Gammaglobulin

A Preliminary Study with ^{131}I labelled Gammaglobulin

By

G. Birke, S.-O. Liljedahl, B. Olhagen, L.-O. Plantin and S. Ahlinder

In the last 10—15 years the concentration of gammaglobulin in serum has been intensively studied by means of various methods (Tiselius' free-boundary electrophoresis, paper electrophoresis, and other techniques). These studies have given very valuable information about diverse pathological conditions. It is probable, however, that additional important information about the gammaglobulin would be obtained if values were recorded for its amount in blood and tissues, as well as for its breakdown, distribution and synthesis. In the last few years methods have been developed for the careful labelling of proteins with ^{131}I and so it has become possible to analyse these parameters in detail.

There are however very few reports on the use of gammaglobulin labelled by non-destructive procedures in studies of the catabolism of gammaglobulin in normal and in pathological conditions. This paper is a preliminary account of data from 10 control cases and from more than 30 patients with various disturbances in the catabolism, distribution or synthesis of gammaglobulin. The results relating to gammaglobulin disturbances in extensive burns will be reported separately (19) as will studies concerning rheumatoid arthritis (27).

Material

The control series consists of 10 patients (cases 1—10) 7 women and 3 men whose ages ranged from 18 to 63 years and who had been admitted to the medical clinic for investigation of disorders of no significance in relation to this study as, for instance, headache and mild diffuse nervous symptoms. All the controls had normal values for serum-protein and normal electrophoretic data, with the exception of case 5 in which the gammaglobulin value was slightly higher than normal.

Acute infections and acute arthritis

A 51-year-old man (case 11) with extensive furuncles in the gluteal region and 3 patients (cases 12, 13, 14) with relatively acute arthritis of varying aetiology were studied.

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Table I. Findings in 10 control subjects

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Extravascular γ-globulin (g)	Total γ-globulin (g)	Half-life ($T/_2$)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
1	41	63	7.1	1.2	32.5	41.3	73.8	16	5.3	1.7	0.026
2	51	79	7.5	1.3	43.3	50.8	94.1	16	4.9	2.1	0.027
3	63	81	8.0	1.6	57.6	70.4	128.0	18	4.4	2.5	0.031
4	18	48	8.0	1.2	32.2	39.4	71.6	16	4.2	1.4	0.029
5	21	68	7.9	0.9	27.7	33.2	60.9	19	5.2	1.4	0.021
6	47	73	7.7	1.1	46.2	63.8	110.0	17	3.6	1.5	0.020
7	40	68	6.7	1.0	24.0	36.0	60.0	22	3.4	0.8	0.012
8	47	58	8.8	0.9	28.7	41.3	70.0	26	7.2	2.1	0.036
9	57	76	8.5	1.2	34.0	29.0	63.0	14	5.8	2.0	0.026
10	44	61	6.9	1.2	31.1	31.1	62.2	13	4.4	1.4	0.023
		Mean	7.5	1.2	35.7	43.6	73.4	18	4.8	1.7	0.025
		Range	(6.7–8.5)	(0.9–1.6)	(24.0–57.6)	(29.0–70.4)	(60.0–128.0)	(13–26)	(3.4–7.2)	(0.8–2.5)	(0.012–0.036)

serum was done before the tracer study and at intervals of at the most 1 week, so that 3 to 4 analyses of this type were obtained for each patient. Accordingly the results relating to the gammaglobulin concentration in serum, which are used in the estimations of the catabolism, were as rule obtained by the free boundary electrophoretic method of Tiselius.

Preparation of ^{131}I-gammaglobulin and methods of determination

Commercial gammaglobulin (AB Kabi, Stockholm) was labelled by technique developed by McFarlane (23) with minor modifications. Purification of the product was made by gel filtration (4). We also prepared autologous gammaglobulin from the myeloma patients by method developed by Björling utilizing DEAE-Sephadex (7).

The content of free ^{131}I was checked by dialysis and was always below 1 %. Paper electrophoresis control showed that the ^{131}I gammaglobulin behaved as normal human non-radioactive gammaglobulin. Ultracentrifuge analysis showed that to 93 % the commercial gammaglobulin used had the normally found sedimentation constant S°_{w} of 6.85. In 7 % the sedimentation constant was 10.75. With free electrophoresis using 1 % solution in veronal buffer of pH 8.6, ionic strength 0.1 only one homogenous component with a migration rate equalling that of the gammaglobulin was registered, however. After the commercial gammaglobulin had been iodinated, the same sedimentation constants with exactly the same percentage distribution as before the labelling were found.

As regards the administration of isotope, determination of radioactivity in plasma, urine, faeces, organs and gastrointestinal juice, the paperelectrophoretic studies, and the measurements of thyroid uptake, detailed account of the procedures has been given in an earlier paper (33).

The method for collecting gastrointestinal juice and the procedure used in the analysis of these specimens were also described in the abovementioned earlier publication. The thyroid gland was in all the investigated cases blocked with Lugol's solution (33). Immunoelectrophoresis and precipitation by Ouchterlony (28) method of intestinal secretion was kindly performed for us by Ass. Prof. R. Klaesner King Gustaf V Research Institute. The

Systemic lupus erythematosus (S.L.E.)

Two women aged 39 (case 15) and 62 (case 16) with typical signs of S.L.E., the former with fever and marked joint-symptoms, the latter in a quiescent phase were investigated.

Myelomatosis

A 44-year-old woman (case 17) with a suspected myeloma was investigated. A confident clinical diagnosis could not be established however as the aspirated sample of sternal marrow showed only plasma-cell proliferation without definite metaplasia and there was no radiographical evidence of skeletal destruction but the patient's serum showed an electrophoretogram typical of myeloma with high concentration of an abnormal gamma-component ('paraprotein')

A 71-year-old woman (case 18) has been studied with a pattern typical for myelomatosis on free electrophoresis. Bence-Jones protein in the urine and multiple skeletal destructions in the skull, lumbar and thoracic spine.

A 67 year-old man with typical signs of myelomatosis. There was markedly increased plasma-cell proliferation in the sternal marrow X-ray showed multiple skeletal destructions and in the patient's serum there was a high concentration of an abnormal homogeneous gamma 2-component

Cirrhosis of the liver

Five patients (cases 20—24) with typical liver cirrhosis were studied. One of them, a 59-year-old man (case 20) had 2 years earlier undergone a portal-shunt operation the other patient (case 21) had marked ascites. Two men aged 44 and 57 years (cases 22 and 23) had alcoholic cirrhosis and oesophageal varices. During the investigation one of them (case 22) had signs of small haemorrhages intermittently from the gastro-intestinal tract.

Ulcerative colitis

Eight patients (cases 25 27—33) with ulcerative colitis in various clinical stages were investigated. The degree of severity is seen in table VI One patient (case 26) with ileitis was also studied

Nephrotic syndrome

A 56-year-old man (case 34) with chronic nephritis in a nephrotic phase was investigated He had slight haematuria of many years duration, moderate proteinuria, and hypertension. For the last year he had markedly increased proteinuria and hypoalbuminaemia as well as hypercholesterolaemia.

Hypogammaglobulinaemia

A 72 year-old man with chronic lymphatic leukaemia and hypogammaglobulinaemia was investigated (case 35 in table VII) A 42-year old man (case 36) had a long history of emaciation with diffuse abdominal disorders and steatorrhoea, resulting from pancreatic cancer accompanied by hypogammaglobulinaemia diagnosed at the routine examination. There was no history of susceptibility to infection.

Case 37 was a 27 year-old woman with histologically verified sarcoidosis of a few years duration, involving mainly the lymph-nodes, particularly in the neck. Since 1959 she had been highly susceptible to infections and had, for instance, had severe bronchopneumonia on a few occasions and the hypogammaglobulinaemia was diagnosed in 1960. Continuous replacement therapy with gammaglobulin improved her condition.

Methods

Serum-proteins

Total serum-protein concentration was determined by Kjeldahl's method. Duplicate determinations were made and the mean was taken.

Paper electrophoresis of the serum-proteins was carried out in veronal buffer (pH 8.6, ionic strength 0.1) for 16 hours with 3 mA per strip. Free electrophoresis by the Thelius technique was applied in all the cases (26) Veronal buffer of pH 8.6 and ionic strength 0.1 was used in an LKB apparatus (LKB-produkter Stockholm)

In the reported estimations of the breakdown and distribution of gammaglobulin the paper-electrophoretic analyses were not used. They merely served the purpose of checking that the relative gammaglobulin situation did not change during the isotope studies. In all the cases paper-electrophoretic analysis of

It will be seen from fig 1 that there is a relatively rapid fall of the plasma disappearance curve which is soon followed by a steady state. A half time of 16 days is obtained, but, as will be seen from the figure, the curve for retained dose does not run exactly parallel to the plasma-disappearance curve, though the deviation is very slight.

The mean catabolic rate for the gammaglobulin was 4 8 % in 24 hours, which corresponds to a mean value of 1 7 g of gammaglobulin per 24 hours. This gives a mean of 0 025 g per 24 hours per kg. Since equilibrium is present and no change of the intravascular gammaglobulin has occurred the synthesis of gammaglobulin must also be 1 7 g per 24 hours. In none of the normal subjects did the faecal excretion of radioactivity over a week exceed 0 1 % of the injected dose.

I the normal subjects (cases 5—7) in whom the gastric excretion of ^{131}I-gammaglobulin was studied, the elimination rate was low or about 0 001 % of the given dose (69—409 cpm/ml) and only 2—10 % was precipitable with trichloroacetic acid (T C.A.) and phosphotungstic acid (P W A.) whereas the rest could be precipitated with $AgNO_3$ This can be estimated to correspond to less than 0 03 g of gammaglobulin per 24 hours, calculating with secretion of 1,000 ml of gastric juice per 24 hours. In 3 normal subjects (cases 5—7) the elimination of ^{131}I-gammaglobulin via the jejunum was also analysed and the collected intestinal juice showed an activity of less than 0 001 of the given dose. Only 0—6 7 % were precipitable with T C.A. plus P W.A. whereas the est could be precipitated with $AgNO_3$. This shows that gammaglobulin cannot be demonstrated with certainty by this method since the precipitable amounts of activity are so small that no significance can be attributed to them. For the same reason neither gastric nor intestinal juice could be studied by paper-electrophoresis. However by immunoelectrophoresis of intestinal juice, gammaglobulin in small amounts could be demonstrated in all the cases. Albumin could also de demonstrated in all the cases.

Analyses of distribution

Table II shows the distribution of radioactive gammaglobulin as a percentage of the injected dose, corrected to the time of the autopsies. The recorded results refer only to organs for which the exact weight could be obtained but the results for some of the other organs will also be discussed.

It is evident from table II that in all the organs, except for the thyroid, the percentages tend to decrease with increasing length of time between injection and death. The activity expressed as per cent of injected dose (table II) is high in the heart the lungs, infected part of the lung, liver and spleen initially and the gastrointestinal tract but relatively low in the other organs.

The thyroid gland and the kidneys are exceptional, with the uptake and excretion, respectively of the liberated ^{131}I In patients nos. 38, 39 41 42 and 43 in whom the thyroid gland was blocked with iodine before the injections of ^{131}I-gammaglobulin, the activity over the thyroid was of a different order in comparison with the non-blocked cases. The activity in the kidneys was much higher in the patients who died 1 1/2 to 2 days after the injections than in those who died 4 to 6 days after the injections.

The results shown in table II are of course dependent on the weights of the

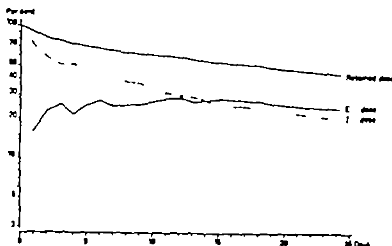


Fig 1 Normal subject with plasma disappearance curve, (I. V dose) retained dose and extravascular dose (E. V dose).

gammaglobulin catabolism was calculated both by the method of Campbell et al. (8) as described by Veall and Vetter (31) and by Matthews's (21) method. A system with four pools was used as model for the calculations as described by Matthews (loc cit) Patients who did not show a constant catabolic rate during the period of investigation were excluded.

Definitions Half-life denotes the time it takes for the intravascular amount of the tagged protein in question to decrease to half its value after the semilogarithmically plotted activity has become a straight line.

Catabolic rate Breakdown of protein expressed as grams or per cent of intravascular pool per 24 hours.

Steady state is a state when pool sizes are constant and synthesis equals breakdown

The designation *pool* is used to describe not a physically or physiologically limited space but has to be conceived rather as a metabolic condition of the protein.

Postmortem analyses of the distribution of gammaglobulin in the body

In 8 patients (cases 38—45) who died, injections of ^{131}I-gammaglobulin in usual doses had been given at varying times before death the times between injection and death are set out in table II At the autopsies the radioactivity in all the organs was measured. The analyses were made on parts that had been freed from as much blood as possible. The total weight was recorded for every organ that could be weighed in its entirety The total radioactivity in an organ *was then estimated* and calculated as a percentage of the injected dose.

Case 38 was a 68-year-old man who died of encephalomalacia and bronchopneumonia. Case 39 was a 64-year-old man who died of cardiosclerosis with heart insufficiency and bronchopneumonia. Case 40 was a 57 year old man who had gastric cancer with metastasis. Case 41 was a 63-year-old woman with malignant lymphogranulomatosis. In case 42, a 64-year-old man, autopsy revealed extensive encephalomalacia and a bleeding duodenal ulcer In cases 34—45 the patients had extensive burns, in case 43 complicated with bronchopneumonia, in case 45 by sepsis.

Results

The control series

The results for the controls are set out in table I

It will be seen from table I that the mean value for serum-gammaglobulin was 1 2 g/100 ml. The intravascular pool averaged 35 7 g and the extravascular pool 43 6 g The ratio extravascular/intravascular gammaglobulin was 1.2 The body's total content of gammaglobulin can be estimated to average 79 4 g The half life of ^{131}I-gammaglobulin determined by the disappearance of ^{131}I-gammaglobulin from plasma averaged 18 days.

Table III. Findings in infections and acute arthritis

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half-life ($T^1/_2$)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
11	51	69	8.5	1 4	57.9	12	10.7	6.2	0.090
12	58	87	8.4	1.8	87 4	15	4 8	4.2	0.048
13	20	70	7 7	1 4	51.8	15	5.1	2.6	0.037
14	18	76	8.8	1.6	56.2	13	3.7	2.1	0.028

Table IV Findings in 2 cases of systemic lupus erythematosus (nos. 15 and 16) and of myelomatosis (nos. 17—19)

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half-life ($T^1/_2$)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
15	59	47	8.2	2.5	91.8	10	10.1	9.3	0.198
16	62	59	9.6	3.2	98.0	7	10.4	10.2	0.174
17 (homologous γ-globulin)	44	67	9.3	2.4	83.3	23	4.7	3.9	0.058
17 (autologous γ-globulin)	44	67	9.7	3.1	104 6	21	4.7	4.9	0.073
18 (homologous γ-globulin)	71	64	12.0	5.6	175.9	10	9.1	16.0	0.252
18 (autologous γ-globulin)	71	64	10.6	4.2	132.5	–	11.4	15.1	0.236
19 (homologous γ-globulin)	68	63	13.0	8.8	537.3	4	19.1	64.4	1.022
19 (autologous γ-globulin)	68	63	14.0	7.8	289 0	4	18.6	55.8	0 886

Only paper-electrophoresis.

those cases in which twofold increase of the intravascular pool was noted. The turnover per 24 hours, expressed both as a percentage nd as g per 24 hours, is in most cases raised in comparison with that in the normal subjects.

Systemic lupus erythematosus

The results for the 2 cases of S.L.E. are recorded in table IV It will be seen from the table that the gammaglobulin concentration as well as the intravascular pool are greatly increased. Both the per

Table II Analysis of distribution of ^{131}I γ-globulin as a percentage of injected dose

Case no.	38	39	40	41	42	43	44	45
Time in hours between injection and death	52	44	25	19	85	96	92	144
Heart	–	2.4	0.9	1.3	–	0.8	0.2	0.2
Lung	–	–	6.6	4.5	4.2	6.9	2.9	1.7
Infected lung	19.3	16.0	–	–	–	–	–	–
Liver	16.2	14.2	3.7	6.2	3.8	2.8	0.9	1.3
Spleen	1.6	1.8	0.3	2.9	1.0	0.2	<0.1	0.1
Kidney	7.0	4.3	1.9	1.8	1.0	0.4	0.2	0.3
Thyroid	–	<0.1	6.8	0.1	1.7	0.2	3.4	17.1
Stomach	4.0	4.1	0.5	1.0	0.6	0.5	0.5	0.9
Gastric contents	–	–	4.0	–	–	–	0.4	2.7
Duodenum	0.5	0.2	0.6	–	0.2	–	–	–
Ileum } Jejunum	5.7	3.4	2.6	0.6 0.4	0.7	10.1	1.0	1.3
Contents	–	–	3.8	–	–	–	1.1	–
Colon	6.6	9.1	–	2.2	0.9	–	0.3	–
Pancreas	<0.1	0.7	0.4	0.2	<0.1	0.1	<0.1	<0.1
Pituitary	–	–	<0.1	–	–	<0.1	–	–
Brain	–	1.0	1.3	0.4	0.3	0.4	<0.1	0.3
Pericardial fluid	–	–	0.2	–	0.1	–	–	0.3
Pleural fluid	–	–	2.3	–	–	–	–	–
Oesophagus	–	–	–	0.2	–	–	–	–
Adrenals	0.4	<0.1	–	0.2	<0.1	<0.1	–	<0.1
Bile	–	–	–	–	–	<0.1	–	<0.1
Ovaries	–	–	–	–	–	–	<0.1	0.1
Urinary bladder	0.3	–	–	–	<0.1	–	–	<0.1

different organs. To eliminate this the activity has also been calculated as a percentage of the injected dose per gram tissue. A high activity is then still observed in the heart lungs liver spleen (initially) and the gastrointestinal tract the adrenals infected tissue and burned skin A moderately high activity was recorded in the exudates, pancreas bone marrow and urinary bladder A relatively low activity was observed in the brain liquor the muscles normal skin and subcutis. These last mentioned results calculated per gram of tissue are influenced by the blood content in the different organs as the activity in all cases was definitely higher in the blood than in the organs This question is therefore under study The data of this investigation as well as a more detailed description of the results from the autopsies will be published elsewhere (1)

Acute infections and arthritis

The results relating to acute mild infections and to 5 cases of relatively acute joint-affections are recorded in table III. Results from rheumatoid arthritis are not reported here, as data concerning this disease will be published separately (27)

It will be seen from table III that two of the patients with infections had moderately raised levels of serum-gammaglobulin. The absolute content of intravascular gammaglobulin shows moderately to relatively markedly elevated levels in

Table III. Findings in infections and acute arthritis

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half-life ($T/_2$)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
11	31	69	8.5	1.4	57.9	12	10.7	6.2	0.090
12	38	87	8.4	1.8	87.4	15	4.8	4.2	0.048
13	20	70	7.7	1.4	51.8	15	5.1	2.8	0.037
14	18	76	8.8	1.6	56.2	13	3.7	2.1	0.028

Table IV. Findings in 2 cases of systemic lupus erythematosus (nos. 15 and 16) and of myelomatosis (nos. 17—19)

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half-life ($T/_2$)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
15	39	47	8.2	2.5	91.8	10	10.1	9.3	0.198
16	62	59	9.6	3.2	98.0	7	10.4	10.2	0.174
17 (homologous γ-globulin)	44	67	8.3	2.4	83.3	23	4.7	3.9	0.058
17 (autologous γ-globulin)	44	67	9.7	3.1	104.6	21	4.7	4.9	0.073
18 (homologous γ-globulin)	71	64	12.0	5.6	173.9	10	9.1	16.8	0.252
18 (autologous γ-globulin)	71	64	10.6	4.2	152.3	—	11.4	15.1	0.236
19 (homologous γ-globulin)	68	63	13.0	8.8	337.3	4	19.1	64.4	1.022
19 (autologous γ-globulin)	68	63	14.8	7.8	299.0	4	18.6	55.8	0.886

Only paper-electrophoresis.

those cases in which a twofold increase of the intravascular pool was noted. The turnover per 24 hours, expressed both as a percentage and as g per 24 hours, is in most cases raised in comparison with that in the normal subjects.

Systemic lupus erythematosus

The results for the 2 cases of S.L.E. are recorded in table IV. It will be seen from the table that the gammaglobulin concentration as well as the intravascular pool are greatly increased. Both the per

Table V Findings in 5 cases of liver cirrhosis

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half life (T½)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
20	59	90	7.3	2.1	80.0	19	4.2	3.4	0.038
21	65	66	7.7	1.5	52.0	25	7.4	3.8	0.058
22	44	78	7.7	1.4	60.7	14	9.1	5.5	0.071
23	48	83	8.0	1.9	98.1	13	6.3	6.2	0.075
24	47	105	6.5	2.7	141.5	16	7.3	10.3	0.098

centage and the absolute catabolism are markedly raised being 5 to 6 times higher than in the normal subjects.

Myelomatosis

Table IV also shows the analytical results for autologous and homologous gamma globulin in 3 cases of myelomatosis The *catabolism expressed as g per 24 hours is* moderately to markedly raised partly because of the greatly increased intra vascular pool. The catabolism of autologous and homologous gammaglobulin was almost identical in the 3 investigated cases.

Cirrhosis of the liver

Table V shows the results from 5 cases of hepatic cirrhosis of varying degrees of severity

The serum-gammaglobulin level is in 3 cases moderately and in 2 cases markedly raised. In all the cases the intravascular pool *is greatly increased* in 3 cases being 3 to 4 times that found in health. It should be noted that one patient (case 22) had minor gastrointestinal haemorrhages. The percentage catabolism in the first case is slightly lowered but because of the high intravascular gammaglobulin content the catabolism expressed as g per 24 hours will be insignificantly raised. In the last named 3 cases the gammaglobulin catabolism is distinctly raised.

Ulcerative colitis and enteritis

The results from the 9 investigated cases of intestinal disorders are set out in table VI

It will be seen from table VI that the serum gammaglobulin level is normal or in 3 cases, slightly raised. In 6 of 9 cases, the absolute amount of intravascular gammaglobulin is normal. The break down of gammaglobulin expressed as a percentage and g per 24 hours is slightly to moderately raised in *all* cases, except for case 26 in which the values are very high

In 6 of the 9 cases the faecal excretion of radioactivity could be determined exactly It was very markedly raised and values between 4 4 and 16 % of the injected dose were obtained over 10—15 days of collection In the remaining 3 cases no exact figures for the whole period of collection can be given but the data that could be obtained show clearly raised values. These observations prove that there is a considerable leakage of

Table VI. Findings in 8 patients with ulcerative colitis, 1 patient with ileitis (no. 26) and 1 patient with nephrotic syndrome (no. 34)

Case no.	Age	Weight	Severity of disease	Blood in faeces	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half-life ($T^1/_2$)	Degradation (%/day) Faecal excretion excluded	Degradation (%/day) Faecal excretion included	Degradation (g/day) Faecal excretion excluded	Degradation (g/day) Faecal excretion included	Degradation (g/day/kg) Faecal excretion excluded	Degradation (g/day/kg) Faecal excretion included
25	18	63	++	–	9 7	1.6	36.9	11	4.0	6.0	2.3	3.4	0.037	0.054
26	44	77	+++	–	5.2	1.0	54.7	7	12.7	–	7.2	–	0.094	–
27	22	54	++	–	6.6	1 4	35.2	10	10.5	14.8	3.7	5.2	0.069	0.096
28	57	69	++	–	8.0	1.8	79.1	11	5.0	9.6	4.0	7.6	0.058	0.111
29	47	53	(+)	–	7.3	1.2	42.5	17	4.7	–	2.0	–	0.038	–
30	51	50	+++	++	6.8	1.1	38.6	9	10.3	11.3	4.0	4.4	0.079	0.087
31	20	50	+++	–	9 0	2 0	43 6	8	4.2	10.4	1.8	4.5	0.036	0.090
32	51	54	++	++	6.2	1.2	42.2	12	8.0	20.5	3.4	8.7	0.063	0.162
33	23	48	(+)	–	5.8	1.2	29.2	5	12.5	–	3.7	–	0.078	–
34	58	68	–	–	3.9	0.9	28.8	5	8.5	13 8	2.4	4.0	0.035	0.058

With precipitable urinary activity

Table VII. Findings in 3 cases of hypogammaglobulinaemia

Case no.	Age	Weight	Total protein (g/100 ml serum)	γ-globulin (g/100 ml serum)	Intravascular γ-globulin (g)	Half-life ($T^1/_2$)	Degradation (%/day)	Degradation (g/day)	Degradation (g/day/kg)
35	72	59	7 1	0.5	21.8	31	2.4	0.52	0.0088
36	42	43	6.7	0.4	8 2	34	3.9	0.32	0.0075
37	26	52	6 6	0 6	13.7	34	3.3	0.45	0.0086

gammaglobulin. In 2 cases intestinal bleeding as indicated in the table, possibly contributes to this leakage. In the calculation of the values for gammaglobulin catabolism the faecal excretion of radioactivity was excluded (table VI). If the faecal loss is included in the reckoning still higher catabolic values will of course be obtained (table VI). In case 31 the activity in the colon could be measured after colectomy. The activity in the colon was 4.3 % of the injected dose, which is a higher value than that obtained in the autopsy cases without colitis with an equally long interval between the injection and the radioactivity studies.

Nephrotic syndrome

In case 34 the value for intravascular gammaglobulin was relatively low (table VI) A slightly raised gammaglobulin catabolism is noted If the protein bound urinary activity which was one third of the total activity is included in the calculation a distinctly raised gammaglobulin catabolism will be obtained

Hypogammaglobulinaemia

The results from the investigated cases of hypogammaglobulinaemia are shown in table VII

The patient with lymphatic leukaemia (case 35) had low values for intravascular gammaglobulin, and the half time of the gammaglobulin was markedly prolonged The percentage and the absolute catabolism was greatly reduced

In the patient with hypogammaglobulinaemia and cancer (case 36) and in the patient with sarcoidosis and hypogammaglobulinaemia (case 37) the gamma globulin pattern was of the same type as that in the patient with leukaemia

Discussion

In the last few years studies on the distribution and catabolism of ^{131}I-*albumin* have given rise to discussions concerning the possible limitations of such investigations. Now it seems on the whole to be agreed that ^{131}I albumin labelled by non destructive procedures, behaves as non radioactive albumin except for the well known fact that the ^{131}I thyrosin liberated at the catabolism of the albumin is not concerned in the resynthesis of proteins. There seems also to be unanimity of opinion as regards the limitations and possibilities that attach to the generally employed methods of calculation.

In studying the distribution and catabolism of *gammaglobulin* in health and, particularly in pathological conditions by means of isotopically labelled proteins, we are faced with problems, some of which are common to all protein studies, while others are specific for gammaglobulin

The general aspects have been dealt with in the foregoing, and only the special problems relating to the gammaglobulins will be discussed here. In contrast to albumin gammaglobulin is not a homogenous protein but as is well known consists of a group of closely related proteins. If this complex is injected there will be a rapid initial fall of the plasma disappearance curve, suggesting a metabolic heterogeneity in the gammaglobulins (10) If the macroglobulin with a sedimentation constant of 19 S is eliminated from the preparations, the same rapid fall of the plasma-disappearance curve does not occur Cohen and Freeman obtained a half-life of 18 days for the pooled gammaglobulin. The homogenous gammaglobulin with a sedimentation constant of 6 10—6 75 S_{20} gave a half life of 21 to 26 days with a catabolic rate of 4 to 6 1 % Higher catabolic rates and shorter half lives have earlier been reported for non-defined gammaglobulins (11 12 14 25 30) The possible explanation of these different values is that partly denaturated proteins were studied. In the present investigation a mean catabolic rate of 4 8 % with a mean half-life of 18 days was obtained for the control subjects (93 % of the studied gamma globulin had a sedimentation constant of about 7 S) The estimation of the half life from the plasma-disappearance curve involves great difficulties, however An exact determination of the half-life is always difficult in isotope studies, when

we are concerned with half-lives as long as those for gammaglobulin. Lewallen et al. (18) and Mills et al. (24) in accordance with others who are working with such problems, found that they obtained a longer half-life when they measured on a later part of the curve and that the later they measured the longer became the half life. To ensure comparable results, more exact and standardized principles of calculation are necessary as is an exact specification of the character of the studied gammaglobulin (or gamma globulins)

In view of these disadvantages and the difficulty of defining unequivocally the term half-life, it would perhaps be better to attach less importance to this term and in future estimations of gammaglobulin catabolism, to give emphasis to the catabolic rate expressed as a percentage and as g per 24 hours, if required in relation to the patients weight.

In studies of patients who are not in a steady state (synthesis = breakdown) at the examinations, special problems arise. In conditions attended with a markedly raised gammaglobulin production the gammaglobulin pool, can for instance, increase by a few per cent per 24 hours, which might cause a considerable error in the turnover studies. In the present study we have tried to offset this source of error by repeated serum-gammaglobulin analyses, in some cases combined with repeated blood-volume analyses.

The distinction between injected homologous and autologous gammaglobulin is another important factor If, for instance, hypergammaglobulinaemia is studied a crucial question is whether the injected normal gammaglobulin is catabolised in the same way as that produced by the diseased body itself. In, for instance, myeloma, cirrhosis of the liver disseminated lupus erythematosus, and rheumatoid arthritis gammaglobulins are produced in an increased amount, but their relations to the normal gammaglobulins have not been made finally clear Immunological studies in myeloma, however suggest qualitative deviations in some cases (15) and similar observations have been made with respect to the macroglobulin in rheumatoid arthritis (the rheumatoid factor) Lippincott et al. (20) consider that it is the protein pattern of the recipient and not the injected protein that will determine the outcome of the gammaglobulin studies. McFarlane (22) has reported results that favour this view as have Berson and Yalow (3) Our results with homologous and autologous gammaglobulin suggest that they are catabolised in a representative way even in diseases with an abnormal gamma globulin pattern. This important problem merits further research and is at present being studied by us.

In many earlier albumin studies and in the few gammaglobulin analyses it has been pre-supposed and regarded as a fact, mostly without further analyses, that the retained-dose and the plasma-disappearance curves run parallel. Lewallen et al. (18) and Wetterfors et al. (32) have shown that this does not hold true for albumin studies in normal subjects, if the retained-dose curve is obtained by subtracting cumulative urine activity from the injected dose. Matthews (21) however has reported parallel curves in animal experiments after whole-body measurements. Birke et al. (6) have also obtained parallel curves by whole-body measurements, but if the retained-dose curve is calculated in the traditional way there is some discrepancy in these experiments as well. The reason for the slight lack of parallelism may be that so far it has not

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In studies of patients who are not in a steady state (synthesis = breakdown) at the examinations, special problems arise. In conditions attended with a markedly raised gammaglobulin production the gammaglobulin pool, can for instance, increase by a few per cent per 24 hours, which might cause a considerable error in the turnover studies. In the present study we have tried to offset this source of error by repeated serum-gammaglobulin analyses, in some cases combined with repeated blood-volume analyses.

The distinction between injected homologous and autologous gammaglobulin is another important factor. If, for instance hypergammaglobulinaemia is studied, a crucial question is whether the injected normal gammaglobulin is catabolised in the same way as that produced by the diseased body itself. In, for instance myeloma, cirrhosis of the liver disseminated lupus erythematosus, and rheumatoid arthritis gammaglobulins are produced in an increased amount, but their relations to the 'normal' gammaglobulins have not been made finally clear. Immunological studies in myeloma however suggest qualitative deviations in some cases (15) and similar observations have been made with respect to the macroglobulin in rheumatoid arthritis (the rheumatoid factor). Lippincott et al. (20) consider that it is the protein pattern of the recipient and not the injected protein that will determine the outcome of the gammaglobulin studies. McFarlane (22) has reported results that favour this view as have Berson and Yalow (5). Our results with homologous and autologous gammaglobulin suggest that they are catabolised in a representative way even in diseases with an abnormal gammaglobulin pattern. This important problem merits further research and is at present being studied by us.

In many earlier albumin studies and in the few gammaglobulin analyses it has been pre-supposed and regarded as a fact mostly without further analyses, that the retained-dose and the plasma-disappearance curves run parallel. Lewallen et al. (18) and Wetterfors et al. (32) have shown that this does not hold true for albumin studies in normal subjects, if the retained-dose curve is obtained by subtracting cumulative urine activity from the injected dose. Matthews (21) however has reported parallel curves in animal experiments after whole-body measurements. Birke et al. (6) have also obtained parallel curves by whole-body measurements, but if the retained-dose curve is calculated in the traditional way there is some discrepancy in these experiments as well. The reason for the slight lack of parallelism may be that so far it has not

Nephrotic syndrome

In case 34 the value for intravascular gammaglobulin was relatively low (table VI) A slightly raised gammaglobulin catabolism is noted If the protein bound urinary activity which was one third of the total activity is included in the calculation a distinctly raised gammaglobulin catabolism will be obtained

Hypogammaglobulinaemia

The results from the investigated cases of hypogammaglobulinaemia are shown in table VII

The patient with lymphatic leukaemia (case 35) had low values for intravascular gammaglobulin and the half time of the gammaglobulin was markedly prolonged The percentage and the absolute catabolism was greatly reduced

In the patient with hypogammaglobulinaemia and cancer (case 36) and in the patient with sarcoidosis and hypogammaglobulinaemia (case 37) the gamma globulin pattern was of the same type as that in the patient with leukaemia

Discussion

In the last few years studies on the distribution and catabolism of ^{131}I-albumin have given rise to discussions concerning the possible limitations of such investigations. Now it seems on the whole to be agreed that ^{131}I-albumin labelled by non destructive procedures behaves as non radioactive albumin except for the well known fact that the ^{131}I thyroxin liberated at the catabolism of the albumin is not concerned in the resynthesis of proteins. There seems also to be unanimity of opinion as regards the limitations and possibilities that attach to the generally employed methods of calculation.

In studying the distribution and catabolism of gammaglobulin in health and, particularly in pathological conditions by means of isotopically labelled proteins, we are faced with problems, some of which are common to all protein studies, while others are specific for gamma globulin

The general aspects have been dealt with in the foregoing and only the special problems relating to the gammaglobulins will be discussed here In contrast to albumin gammaglobulin is not a homogenous protein but, as is well known consists of a group of closely related proteins. If this complex is injected there will be a rapid initial fall of the plasma disappearance curve suggesting a metabolic heterogeneity in the gammaglobulins (10) If the macroglobulin with a sedimentation constant of 19 S is eliminated from the preparations, the same rapid fall of the plasma-disappearance curve does not occur Cohen and Freeman obtained a half-life of 18 days for the pooled gammaglobulin The homogenous gammaglobulin with a sedimentation constant of 6 10—6 75 S_{20} gave a half-life of 21 to 26 days with a catabolic rate of 4 to 6 1 % Higher catabolic rates and shorter half lives have earlier been reported for non-defined gammaglobulins (11 12 14 25 30) The possible explanation of these different values is that partly denaturated proteins were studied. In the present investigation a mean catabolic rate of 4 8 % with a mean half life of 18 days was obtained for the control subjects (93 % of the studied gamma globulin had a sedimentation constant of about 7 S) The estimation of the half life from the plasma-disappearance curve involves great difficulties, however An exact determination of the half-life is always difficult in isotope studies, when

half-life tended to be longer than normal, in accordance with the results reported by Poliwoda and Blaurus (29). Because of the greatly increased intravascular pool the breakdown rate expressed in g per 24 hours will, however, be raised in the cases with a long half-life.

The patients with ulcerative colitis and the patient with enteritis had a high faecal loss of radioactivity which showed good correlation with the clinical picture. The high faecal loss of radioactivity is in most cases not caused by intestinal haemorrhage. If the faecal excretion of activity is excluded at the catabolic measurement, a moderately raised catabolism will be obtained in most cases. If the faecal loss is included the catabolism is distinctly raised in all the investigated cases. In the case of ileitis the catabolic rate was very high, however. This is attributable partly to the markedly increased intravascular pool, which in the rest of the cases was only moderately increased. A high activity was noted in the colon after colectomy which may suggest an increased extravascular distribution and breakdown in ulcerative colitis. This assumption is supported by the shape of the disappearance curve.

Gitlin et al. (15) and Mills et al. (24) recorded a short half-life for patients with the nephrotic syndrome. The same result was obtained in the present study. The short half-life is explained by a distinct protein-bound urinary excretion of radioactivity but also by a percentage increase of the gammaglobulin catabolism, which because of the relatively low intravascular gammaglobulin pool will be only slightly raised if expressed in g per 24 hours. Patients with agammaglobulinaemia have earlier been found to have a prolonged half-life (17, 34, 3..). The patients with hypogammaglobulinaemia also had a distinctly reduced intravascular pool and markedly prolonged half life. The gammaglobulin catabolism in terms of percentage is slightly reduced whereas because of the low intravascular pool, it will be markedly reduced if expressed in grams of catabolised protein per 24 hours. This suggests that in hypogammaglobulinaemia as well the characteristic pattern of reaction is markedly reduced gammaglobulin catabolism as a sign of an 'economy' mechanism of the greatly reduced gammaglobulin pool.

Earlier studies of the serum-gammaglobulin concentration by diverse electrophoretic techniques have given a static picture of the gammaglobulin situation in the body. However these methods of investigation provide no information either about the catabolism or about the synthesis of the gammaglobulin. Studies with isotopically labelled proteins give a more dynamic picture of the protein situation in the body. Such studies have already furnished new data which reveal several types of gammaglobulin patterns. Firstly in diseases accompanied by distinct hypergammaglobulinaemia as for instance disseminated lupus erythematosus, there is, despite the raised serum-gammaglobulin level, markedly increased breakdown, which indicates that these conditions are attended with a gammaglobulin synthesis far higher than could earlier be imagined. An increased gammaglobulin degradation is also observed in myeloma and to a lower degree, in mild infections in which the serum-gammaglobulin level is also raised. Secondly normal values for serum-gammaglobulin are found in, for instance, ulcerative colitis and the nephrotic syndrome, but in these conditions too the gammaglobulin catabolism is increased. This is attributed mainly to loss of gammaglobulin into

been possible in man to carry out whole-body measurements or continue the investigation over a sufficiently long time. In gammaglobulin analyses this question has not yet been considered Cohen and Freeman (10) report slightly deviating curves in normal subjects but do not discuss the problem further In our study the curves obtained for retained dose and plasma-disappearance in the normal subjects also deviated slightly In the pathological cases this tendency was more marked For this reason the size of the extravascular gammaglobulin pool in the pathological conditions can not be discussed until further data are available.

Cohen and Freeman measured the gammaglobulin catabolism in a few normal subjects and reported identical results by four principles of calculation which suggests that the slight deviation in the course between retained-dose and plasma-disappearance curves does not significantly influence the results.

Despite the limitations and the yet unsolved problems relating to the gammaglobulin studies, some conclusions can justifiably be drawn from the obtained results both in the pathological cases and in the normal subjects Gammaglobulin of 7 S has in the control subjects a mean catabolic rate of 4 8 % which is clearly lower than that of albumin An average of 1 7 g of gammaglobulin is catabolised per 24 hours and the intravascular pool averages 36 g and the extravascular pool 44 g

Gammaglobulin has earlier been demonstrated in secretion from the small intestine by immunoelectrophoresis (2 16) This has been confirmed in the present study in normals, but experiments designed to study semiquantitatively the amount of gammaglobulin that is excreted through the gastrointestinal tract have shown that the loss is negligible.

Analyses of the distribution of ^{131}I gammaglobulin in 8 cases in which the time between injection and death varied showed a strikingly high activity in the lungs, liver and gastrointestinal tract, whereas in the spleen with its high content of blood the activity was lower in most cases As will be discussed separately (19) infected tissue and burnt skin showed high activity The first mentioned results have been confirmed by experimental investigations on animals and are the object of continued analysis (1)

Thus in contrast to what has been shown for albumin (5 33) it seems improbable that gammaglobulin is to any great extent degraded after leakage into the gastrointestinal tract. The high figures for activity in the lungs, gastrointestinal tract, liver and infected tissue may possibly be of some significance in this connection. Cohen et al. (9) have also drawn attention to the importance of the liver in the catabolism of gammaglobulin

In mild acute infections the intravascular gammaglobulin pool is increased with a raised turnover rate both in terms of percentage and calculated as g per 24 hours. This suggests an increased gammaglobulin synthesis. The patient who had S.L.E. in a quiescent phase and in whom, judging by the blood-volume analyses and the repeated paper-electrophoretic studies, equilibrium between synthesis and catabolism was present had a markedly increased intravascular gammaglobulin pool but with a five-fold rise of the gammaglobulin break-down rate. This suggests a greatly increased rate of gammaglobulin synthesis. It is evidently much higher than the raised serum gammaglobulin values suggest.

Patients with cirrhosis of the liver had a distinctly increased intravascular gammaglobulin pool. In 2 of the cases the

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the intestine and the kidneys respectively The third pattern is seen in agammaglobulinaemia and hypogammaglobulinaemia in which the catabolism is distinct ly reduced

Summary

7 S gammaglobulin was labelled with ^{131}I and the catabolism of this ^{131}I gammaglobulin was studied in 10 control subjects A mean catabolic rate of 4 8 % per day was obtained This corresponds to a breakdown of 1 7 g of gammaglobulin per day or 0 025 g per kg body weight The intravascular gammaglobulin pool averaged 36 g and the extra vascular pool 44 g

Gammaglobulin was demonstrated electrophoretically in secretions from the small intestine in accordance with earlier investigations, but the amount of gamma globulin excreted through the gastrointestinal tract was negligible, as examined by the method which has earlier shown that in health albumin is to a great extent lost into the gastrointestinal tract

The distribution of gammaglobulin was studied in 8 autopsy cases. Varying *times had elapsed between the injection* of gammaglobulin and death. In all the cases a high activity was noted in the lungs and the liver as well as in the gastrointestinal wall whereas the spleen with its high content of blood showed strikingly low activity Infected tissue had high radioactivity

Mild infections were accompanied by an increased intravascular gammaglobulin pool and a moderately raised de gradation rate. In systemic lupus erythematosus the intravascular pool was markedly increased *and the breakdown* of gammaglobulin was 5 to 6 times that in health Myelomatosis was accompanied by an increase *in catabolism* of varying degrees, the turnover rate being the same for autologous and *homologous protein.* In cirrhosis of the liver there was an increase in the *gammaglobulin pool* of varying degrees, with a moderately raised catabolic rate Patients with ulcer ative colitis and nephrotic syndrome had normal values for the intravascular pool and increased catabolism If the losses through the intestine and the kidneys were excluded the catabolism was only moderately raised In *hypogammaglobulinaemia* the catabolic rate was clearly reduced

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Studies on Free and Serum Protein-bound Vitamin B_{12} by the Use of Sephadex G 25 and High Voltage Electrophoresis[1]

By

Kai Lindstrand, Karl-Gustav Ståhlberg, Gösta Ehrenvärd and Åke Nordén

Active chronic myelocytic leukaemia is characterized by a high level of serum vitamin B_{12} (1, 5, 11, 15) in association with an increased spare capacity of the serum for binding vitamin B_{12} (7, 8, 9, 10, 14). The importance of these findings as part of the pathophysiology of chronic myelocytic leukaemia has been debated.

For the study *in vitro* of the vitamin B_{12}-binding capacity of serum and the identification of the serum protein responsible for the binding different methods have been employed. The results have suggested several possibilities for the binding with no great agreement between the findings of different investigators. As this could partly be explained on technical grounds a method has been sought which would allow of simple and gentle handling of the serum proteins.

The present report deals with the problem of separating free and protein-bound vitamin B_{12}. By the use of gel filtration through a column of Sephadex G 25, vitamin B_{12} bound to serum protein was found to pass through the column rapidly while the passage of the non protein-bound vitamin was delayed. This technique had independently been used by Dalsley (5) for the isolation of vitamin B_{12} from sea water.

Material and methods

Vitamin B_{12} labeled with Co^{60} was obtained from the Radiochemical Centre, Amersham, England. The activity of the preparation was examined by microbiological assay in our laboratory with the use of Euglena gracilis z strain and by radioactive measurements through the courtesy of Professor Kurt Lidén, Head of the Hospital Radiophysical Laboratory. The specific activity was found to be 10.9 μC Co^{60} per μg vitamin B_{12}.

Sephadex G 25, Medium, from Pharmacia, Uppsala, Sweden, was used in a column with height of 22 cm and volume of 17.1 ml.

Carbowax (polyethylenglycol compound 20 — M) from the Union Carbide Chemicals

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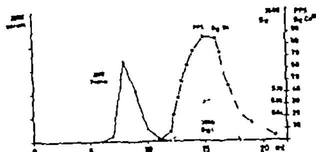


Fig. 2. Optical density measured at 2,800 Å for proteins —— and 13,600 Å for vitamin B_{12} - -. Radioactivity (PPS) emanating from Co^{60} B_{12} ⊙—⊙

II. HIGH VOLTAGE ELECTROPHORESIS OF SERUM INCUBATED WITH Co^{57} B_{12}

0.3 ml serum was incubated with ≈ 4,000 pg Co^{57} B_{12} at 30° C for 60 minutes. The serum was then fractionated by high voltage electrophoresis in pevicon. When the separation was finished imprints were made by covering the pevicon with Whatman paper grade No. 1. The paper was removed and stained with bromphenol blue for protein. The electrophoretic slide was scanned and the radioactivity was recorded automatically (fig. 1).

Normal sera

Radioactivity appeared in regions corresponding roughly to the γ-, the β_1- and the α_1-bands (fig. 3). The radioactivity in the α_1-region was only faint.

Pathological sera

In fig. 4 the radioactivity pattern of a pernicious anaemia serum is shown. The radioactivity in the α_1-region is more marked than in the normal serum. Fig. 5 illustrates the findings in case of chronic myelocytic leukaemia. The radioactivity in the α_1-region is still more marked than in the pernicious anaemia serum.

III. COMPARISON BETWEEN FRACTIONATION BY HIGH VOLTAGE ELECTROPHORESIS AND BY SEPHADEX FOLLOWED BY HIGH VOLTAGE ELECTROPHORESIS

0.6 ml serum was incubated with ≈ 8,800 pg Co^{57} B_{12} as described under II.

A. Half of the serum was fractionated by high voltage electrophoresis in pevicon and studied for radioactivity as described above.

B. The other half of the serum was passed through the column of Sephadex G 25 in the way described under I. The radioactivity of each collecting tube was measured in the well-type crystal. Eluate fractions known from experiment No I to contain serum protein (tubes 6—12 in fig. 2) were pooled and concentrated against carbowax for three hours. The solution was then separated by high voltage electrophoresis and scanned for radioactivity.

This experiment was designed to determine whether the three serum peaks of radioactivity γ, β_1 and α_1, found in the experiments described under II represented free or serum protein-bound radioactive vitamin B_{12}. Serum from a patient with chronic myelocytic leukaemia was used.

Fig. 6 A shows separation by high voltage electrophoresis without previous passage through a column of Sephadex G 25. Radioactivity was found in the γ-, in the β_1 and in the α_1-regions. In fig. 6 B the serum had passed a column of Sephadex G 25 prior to high voltage electrophoresis. The electrophoretically separated serum showed radioactivity in the β_1- and in the α_1-regions to the same extent as in the previous experiment (fig. 6 A). However there was hardly any radioactivity in the γ-region. As shown in experiment No. I the free vitamin B_{12} is delayed by the Sephadex G 25. It may therefore be assumed that the radioactivity in the γ-region emanates from free Co^{57} B_{12} whereas the activity in the β_1 and in the α_1-regions represents the protein-bound portion of the radioactive vitamin added *in vitro*.

IV. DETERMINATION OF THE SPARE CAPACITY OF SERUM FOR BINDING VITAMIN B_{12}

Serum from two healthy persons, one patient with pernicious anaemia and three

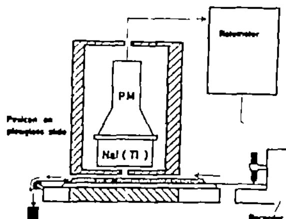


Fig 1 Arrangement for the scanning of the pevicon mass. The plexi-glass is attached to the paper of the recorder and therefore traverses it at the constant speed of 1 cm/min. The distance between the NaI (Tl) crystal and the surface of the pevicon is 15 mm. The slit in the lead shield facing the crystal has a width of 3 mm.

Company was used for the concentration of serum fractions eluated from the Sephadex column.

Eluent barbiturate buffer ionic strength 0 1 pH 8.2

Pevicon C 870 (co-polymer of polyvinyl chloride and polyvinyl acetate) from Fosfat bolaget, Stockholm, Sweden, was used as described by Muller Eberhard (12)

High voltage electrophoresis was performed at 15 V per cm in a 2 mm thick pevicon layer on a plexi-glass slide (45 × 250 mm) at + 4 C for five hours. Barbiturate buffer ionic strength 0 1 pH 8.2 was used.

Serum was obtained from three healthy persons, four patients with pernicious anaemia and five patients suffering from chronic myelocytic leukaemia.

Radioactive measurements

The radioactivity of samples eluated from the Sephadex column was determined in a well-type NaI (Tl) crystal (N 597 Ekco Electronic Ltd) with a diameter of 1 3/4" by 2" long and a central well 11/16" diameter by 1 1/2" deep. The scintillation counter (Ekco N 559) was attached to a set containing a high voltage source, amplifier pulse height analyser and ratemeter (Ekco N 600) The measurements were performed at 750 V with a threshold of 12.5 V and a gate width of 3 V The mean probable error was 1 %. The samples were counted for 15 min. or until stability was attained

Following high voltage electrophoresis the pevicon mass was scanned for radioactivity by an arrangement illustrated in fig 1 A NaI (Tl) crystal (Ekco N 566) with a diameter of 1 1/2" by 1" long was used. The pulses from the scintillation counter were recorded automatically by a Varian recorder The measurements were performed at 750 V with a threshold of 19 V and a gate width of 3 V The amplifier gain was 1 000 and the mean probable error 10

Experimental

I. ELUTION OF VITAMIN B_{12} AND SERUM FROM A COLUMN OF SEPHADEX G 25

0.3 ml serum was run through the Sephadex column at a flow rate of 20 ml per hour and the eluate collected in divided samples of 1 ml each. The absorption of the collected fractions at 2,800 Å was determined in a Beckman DU spectrophotometer

For comparison 0.3 ml of a solution containing 15 μg vitamin B_{12} and 1 000 pg Co^{8} B_{12} was passed through the column. The fractions were examined for radioactivity in the well type crystal and spectrophotometrically at 3 600 Å in a Beckman B spectrophotometer

The results of these experiments are given in fig 2. Spectrophotometrical analysis of the eluate fractions from the Sephadex column gave a peak at 2,800 Å (protein absorption curve) in tube 8 and another peak at 3 600 Å (vitamin B_{12} absorption curve) in tubes 15—16. The radioactivity in tubes No. 1—11 was equal to the background activity Radioactivity above this value was registered in tubes 12—21 with a peak in 15—16, corresponding to the maximum absorption at 3 600 Å for vitamin B_{12}. The experiments demonstrate the rapid passage of the serum and the slow passage of the vitamin B_{12} with a distinct separation between the two solutions.

Table I

Pat. no.	Diagnosis	$UB_{12}BC$ of serum		Serum B_{12} (pg/ml)
		Sephadex G 25 (pg/ml)	Electro-phoresis (pg/ml)	
1	Normal	1,327	1,220	530
2	Normal	1,090	1 068	460
3	PA	2,290	2,260	0
4	CML	8,035	8,363	3,920
5	CML	9,590	9,860	7,300
6	CML	12,370	12,570	5,120

PA = Pernicious anaemia.
CML = Chronic myelocytic leukaemia.

As seen from table I the 2 normal sera showed an unsaturated binding capacity of 1 100—1,300 pg/ml, the serum from case of pernicious anaemia had a binding capacity of 2,300 pg/ml. Finally the three sera from cases of chronic myelocytic leukaemia showed as expected high values ranging between 8,000 and 12,600 pg/ml

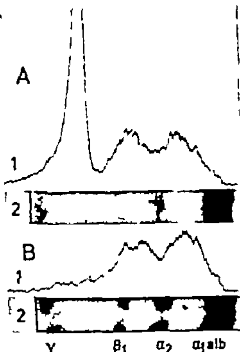


Fig. 6. Chronic myelocytic leukaemia serum separated by high voltage electrophoresis.
A. 1 Record of radioactivity showing activity in approximately the γ- β_1- and α_1-regions.
2 Electrophoretic pattern.
B. 1 Serum from the same patient filtered through column of Sephadex G 25 and then separated by high voltage electrophoresis. Note absence f any peak in the γ-region.
2. Electrophoretic pattern.

Discussion

In serum from normal persons and patients with chronic myelocytic leukaemia, vitamin B_{12} bound in vivo has been found mainly in the α_1- and α_2-globulins (4 13, 16) or in the α_1-globulins only (6) Miller and Sullivan (10) found in vitro-bound radioactive vitamin B_1 mainly in the β_1- and in the α_2-globulins of *normal* serum. In chronic myelocytic leukaemia serum they found the radioactive vitamin chiefly in the α_1- or in the α_2-globulins or in both. Besides, there were relatively large amounts of radioactivity in albumin and γ-globulins in both types of sera. Separation of Co^{*} B_{12}-loaded serum by high voltage electrophoresis in pevicon showed radioactivity in the γ- in the β_1- and in the α_1-regions of normal serum as well as of chronic myelocytic leukaemia serum. Miller and Sullivan (10) supposed that the radioactivity found in the γ-region in their experiments emanated from free radioactive vitamin B_{12}.

In the present studies the gel filtration principle of a Sephadex G 25 column was used for separating free and serum protein-bound vitamin B_{12}. We found that vitamin B_{12} bound to a protein molecule behaves like macromolecul and passes Sephadex G 25 without entering into its *inner* volume. The free (= non-protein-bound) vitamin B_{12} (molecular weight ≃ 1 360)

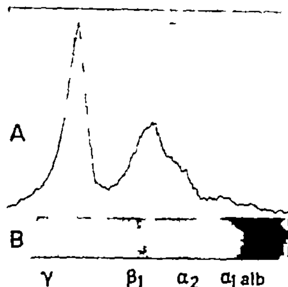


Fig 3 Normal serum separated by high voltage electrophoresis.
A. Record of radioactivity showing peaks corresponding roughly to the γ- and β_1-regions and a faint activity in the α-region
B. Electrophoretic pattern.

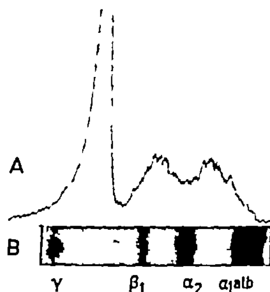


Fig 5 Chronic myelocytic leukaemia serum separated by high voltage electrophoresis.
A. Record of radioactivity showing activity corresponding roughly to the γ- and β_1-regions and also in the α-region.
B. Electrophoretic pattern

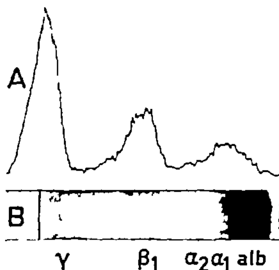


Fig 4 Pernicious anaemia serum separated by high voltage electrophoresis.
A Record of radioactivity with peaks in approximately the γ- β_1 and α-regions.
B. Electrophoretic pattern.

patients suffering from chronic myelocytic leukaemia was examined After incubation with Co^{58} B_{12}, serum from each subject was divided into two portions, A and B.

Portion A was fractionated by high voltage electrophoresis in pevicon and the radioactivity was recorded by scanning

The spare capacity for binding vitamin B_{12} was determined by planimetry of the areas formed by the β_1-, α and γ-radioactivity peaks.

$$UB_{12}BC = \frac{R\,(\beta + \alpha_1)}{\gamma + \beta_1 + \alpha}$$

$UB_{12}BC$ = unsaturated B_{12} binding capacity

R = amount in pg/ml of radioactive vitamin B_{12} added to serum before fractionation.

$\beta + \alpha_1$ = protein-bound vitamin B_{12}.
$\gamma + \beta_1 + \alpha$ = sum of non-protein-bound and protein-bound vitamin B_{12}.

Portion B was run through a column of Sephadex as described above. The spare capacity of serum for binding vitamin B_{12} was formed by the sum of the radioactivity in the eluate fractions known from experiments No. I to contain serum proteins.

When the results of the two procedures were compared, the values for unsaturated vitamin B_{12} binding capacity were found to agree within ± 5 % (table I)

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enters into the inner volume of the column Its passage is therefore delayed and the vitamin becomes separated from the serum proteins When serum incubated with Co^{57} B_{12} was separated by high voltage electrophoresis after passage through Sephadex G 25 radioactivity was found approximately in the β_1 and in the α_1 regions In the γ-region however there was hardly any radioactivity to be detected It may therefore be assumed that the radioactivity of the β_1 and α_1 regions in our experiments corresponds to serum protein bound radioactive vitamin whereas the radioactivity in the γ-region emanates from free vitamin B_{12}.

Filtration of serum incubated with radioactive vitamin B_{12} through a column of Sephadex G 25 was used to determine the spare vitamin B_{12} binding capacity by measuring the protein bound fraction of added radioactive vitamin B_{12}. When values obtained by this method were compared with the amount of radioactive vitamin B_{12} found after high voltage electrophoresis in the α and β_1 regions agreement within ± 5 per cent was found Six sera have been examined and the results are in accord with those reported by others.

Summary

Serum incubated with radioactive vitamin B_{12} was separated by high voltage electrophoresis in pevicon The electrophoretogram was studied for radioactivity by scanning The radioactive vitamin was found approximately in the γ- β_1 and α regions. When serum incubated with radioactive vitamin B_{12} was passed through a column of Sephadex G 25 the passage of free vitamin B_{12} was delayed while serum protein-bound vitamin B_{12} passed rapidly High voltage electrophoresis of serum which had been passed through the Sephadex column showed that the non-protein-bound vitamin corresponded to the radioactivity found in the γ-region on electrophoresis. Filtration through Sephadex was also used for the determination of the spare capacity of serum for binding vitamin B_{12}. Good agreement was found between this method and determinations made by high voltage electrophoresis.

Acknowledgements

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We wish to thank Dr Yngve Naversten for generous help with radioactive measurements.

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Intestinal Absorption of ^{45}Ca in Senile Osteoporosis

By

A. Cantigola, C. Gennari, V Bianchi and R. Guideri

According to Albright (1) a quantitatively deficient formation of protein matrix represents the sole defect in osteoporosis. Such defect leads to a reduction in the rate of formation of new bone, the process of resorption going on at the normal rate.

More recently however Nordin (4 5) indicated chronic calcium deficiency as the determining factor in osteoporosis. According to Nordin, an insufficient dietary calcium intake would lead, in the long run, to prevalence of resorption processes over those of new bone formation.

In his latest works Nordin suggests other possible pathogenetic factors. The negative calcium balance in osteoporosis could be due not only to an inadequate dietary intake, but also to intestinal malabsorption or excessive urinary or fecal excretion of calcium.

In order to establish the rate of intestinal absorption of calcium an oral dose of ^{45}Ca was administered to 13 subjects affected by senile osteoporosis and to 5 normal subjects.

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Material and methods

The normal subjects — two old and three young persons — were the material of our study

The dose of isotope used was exactly as suggested by Blau et al. (2): 50 μc of $^{45}CaCl_2$ of high specific activity equal to 5 mg of calcium. The fasting patients were given the dose orally at 8 a.m., dissolved in 10 ml of a 10 % calcium gluconate solution, equivalent to 88 mg of calcium carrier

Samples of venous blood were taken every 5 minutes for period of thirty minutes, then every 10 minutes during the following hour Other samples were taken one and a half hours, two hours, and four hours after administration of the dose. The degree of radioactivity of samples was established. Counts were expressed as per cent of dose administered per litre of plasma.

Urine was collected every two hours during the six hours following administration of the dose. Feces were taken in a single sample of 72 hours. Radioactivity was determined on each sample. Counts were expressed as per cent of dose administered.

Samples for counting were prepared as follows 1 ml of plasma was added to 3 ml of 10 calcium gluconate solution, equivalent to 26.4 mg of calcium. An excess of 3 % ammonium oxalate was added to precipitate calcium as calcium oxalate, which was collected

Table I Plasma activities (% of dose per litre of plasma)

Pat.	Sex	Age	Minutes											
			5	10	15	20	25	30	40	50	60	90	120	240
Normals														
1	○	31	−	−	+	0.35	0.70	1.00	1.80	2.00	2.15	2.60	2.61	2.40
2	○	62	−	+	0.20	0.60	0.75	1.00	1.50	1.00	2.10	2.30	2.23	1.90
3	♂	76	−	−	+	0.20	0.50	0.70	1.20	1.60	2.00	2.15	2.25	1.95
4	♀	28	−	+	0.30	0.70	1.00	1.20	1.60	1.95	2.30	2.30	2.40	2.18
5	♀	30	−	−	+	0.20	0.60	0.90	1.50	2.10	2.30	2.40	2.35	2.10
Osteoporotic patients														
6	○	59	−	−	−	+	0.30	0.40	0.70	1.00	1.10	1.30	1.30	1.20
7	♀	65	−	−	−	−	−	+	0.40	0.60	0.80	1.05	1.15	1.10
8	♀	75	−	−	−	−	+	0.10	0.30	0.45	0.60	0.80	0.90	0.80
9	♂	47	−	−	−	−	0.20	0.40	0.68	0.90	1.15	1.50	1.60	1.40
10	♀	60	−	−	−	−	+	0.40	0.70	1.00	1.30	1.80	1.90	1.20
11	♀	81	−	−	−	−	+	0.20	0.50	0.80	1.10	1.50	1.70	1.31
12	♀	64	−	−	−	−	+	0.20	0.50	1.00	1.10	1.40	1.36	1.25
13	○	85	−	−	−	−	+	0.10	0.20	0.40	0.45	0.50	0.55	0.50
14	♀	71	−	−	−	−	−	+	0.40	0.70	0.90	1.00	1.10	1.00
15	♀	77	−	−	−	−	+	0.10	0.30	0.45	0.60	0.80	0.90	0.80
16	♀	74	−	−	−	−	−	+	0.20	0.50	0.65	0.90	1.00	0.85
17	♀	72	−	−	−	−	−	+	0.10	0.30	0.45	0.70	0.75	0.60
18	♀	46	−	−	−	−	+	0.10	0.40	0.70	1.00	1.70	1.85	1.60

+ = Small amounts of radioactivity

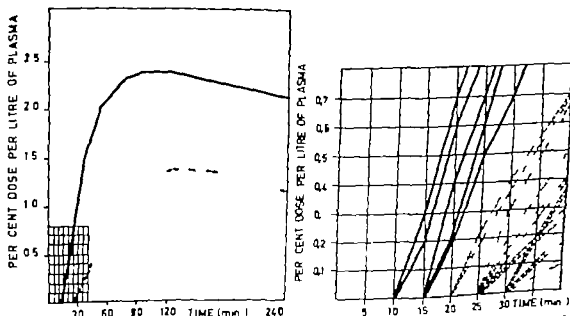


Fig. 1 Plasma activities in normals and osteoporotic patients. Left: the solid line (—) represents the mean of values in normals (5 cases); the dashed line ( ) represents the mean of values in osteoporotic patients (13 cases). Right: plasma activities in the first 30 minutes.

Table II. ^{47}*Ca in urine (% of dose)*

Pat.	Sex	Age	Hours			
			0—2	2—4	4—6	0—6
Normals						
1	♂	31	0.15	0.34	0.49	0.98
2	♂	62	0.13	0.37	0.32	0.82
3	♂	76	0.23	0.46	0.50	1.19
4	♀	28	0.23	0.31	0.47	1.01
5	♀	30	0.27	0.28	0.34	0.89
Osteoporotic patients						
6	♂	59	0.05	0.20	0.20	0.45
7	♀	63	0.02	0.05	0.20	0.27
8	♀	73	0.04	0.10	0.21	0.35
9	♂	47	0.11	0.15	0.16	0.42
10	♀	60	0.16	0.17	0.25	0.58
11	♀	81	0.04	0.14	0.15	0.33
12	♀	64	0.06	0.11	0.22	0.39
13	♂	83	0.01	0.04	0.08	0.13
14	♀	71	0.05	0.11	0.19	0.35
15	♀	77	0.15	0.10	0.12	0.37
16	♀	74	0.10	0.16	0.12	0.38
17	♀	72	0.01	0.08	0.11	0.20
18	♀	46	0.05	0.11	0.15	0.31

on filter paper disk with suction. The activity in the layer on the filter paper disk was determined in shielded sample changer with an endwindow Geiger counter (Tracerlab mod. TGC 2).

All counts were compared to standards prepared in an identical manner from aliquots of the administered dose of ^{47}Ca. A reagent blank was counted as background sample.

Urine samples were added to concentrated hydrochloric acid solution. Acidity was neutralized with NH_3. Calcium gluconate and ammonium oxalate were also added.

Feces were homogenized and weighed. A 10 g sample was ashed in muffle furnace at 600° C. The residue was dissolved in 10 ml of 10 % perchloric acid. Calcium was precipitated in the usual manner.

All counts were corrected for self-absorption using an experimentally determined correction curve. Counting was performed on samples at infinite thickness.

It was unnecessary to correct counts for decay since the standards were always re-

Table III. ^{47}*Ca in stools (% of dose)*

Pat.	Sex	Age	0—72 hours
Normals			
1	♂	31	35
2	♂	62	32
3	♂	76	31
4	♀	28	38
5	♀	30	29
Osteoporotic patients			
6	♀	59	52
7	♀	63	56
8	♀	73	60
9	♂	47	48
10	♀	60	41
11	♀	81	55
12	♀	64	58
13	♂	83	65
14	♀	71	54
15	♀	77	65
16	♀	74	61
17	♀	72	58
18	♀	46	47

Discussion

Radioactivity appears in the plasma of normal subjects, either old or young, within 10—15 minutes after administering the isotope. Plasma radioactivity increases rapidly to a peak within 60 to 90 minutes (table I and fig. 1).

In subjects affected by osteoporosis, radioactivity appears in the plasma only later, not earlier than 20—30 minutes after administration. The increase in radioactivity is much slower and the peak is attained only 120 minutes after administration of the dose.

It is interesting to note the different levels of radioactivity reached by the two groups. In normal subjects, the highest value was between 2 and 2.5 % of the dose per litre of plasma. In subjects affected by osteoporosis the highest value was between 1 and 1.5 % of the dose per

Table I Plasma activities (% of dose per litre of plasma)

Pat.	Sex	Age	Minutes											
			5	10	15	20	25	30	40	50	60	90	120	240
Normals														
1	♂	31	–	–	+	0.35	0.70	1.00	1.80	2.00	2.15	2.60	2.61	2.40
2	♂	62	–	+	0.20	0.60	0.75	1.00	1.50	1.00	2.10	2.30	2.23	1.90
3	♂	76	–	–	+	0.20	0.50	0.70	1.20	1.60	2.00	2.15	2.25	1.95
4	♀	28	–	+	0.30	0.70	1.00	1.20	1.60	1.95	2.30	2.50	2.40	2.18
5	♀	30	–	–	+	0.20	0.60	0.90	1.50	2.10	2.30	2.40	2.35	2.10
Osteoporotic patients														
6	♂	59	–	–	–	+	0.30	0.40	0.70	1.00	1.10	1.30	1.30	1.20
7	♀	65	–	–	–	–	–	+	0.40	0.60	0.80	1.05	1.15	1.10
8	♀	75	–	–	–	–	+	0.10	0.30	0.45	0.60	0.80	0.90	0.80
9	♂	47	–	–	–	–	0.20	0.40	0.68	0.90	1.15	1.30	1.60	1.40
10	♀	60	–	–	–	–	+	0.40	0.70	1.00	1.50	1.80	1.90	1.20
11	♀	81	–	–	–	–	+	0.20	0.50	0.80	1.10	1.50	1.70	1.51
12	♀	64	–	–	–	–	+	0.20	0.50	1.00	1.10	1.40	1.36	1.25
13	♂	85	–	–	–	–	+	0.10	0.20	0.40	0.45	0.50	0.55	0.50
14	♀	71	–	–	–	–	–	+	0.40	0.70	0.90	1.00	1.10	1.00
15	♀	77	–	–	–	–	+	0.10	0.30	0.45	0.60	0.80	0.90	0.80
16	♀	74	–	–	–	–	–	+	0.20	0.50	0.65	0.90	1.00	0.85
17	♀	72	–	–	–	–	–	+	0.10	0.30	0.45	0.70	0.75	0.60
18	♀	46	–	–	–	–	+	0.10	0.40	0.70	1.00	1.70	1.85	1.60

+ = Small amounts of radioactivity

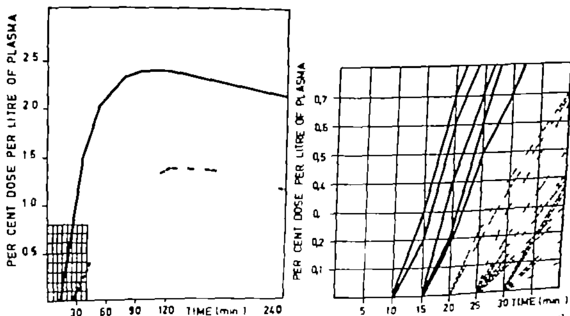


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Table II. ^{45}Ca in urine (% of dose)

Pat.	Sex	Age	Hours 0—2	Hours 2—4	Hours 4—6	Hours 0—6
Normals						
1	o	31	0.15	0.34	0.49	0.98
2	o	62	0.13	0.37	0.32	0.82
3	♂	76	0.23	0.46	0.50	1.19
4	♀	28	0.27	0.31	0.47	1.01
5	♀	30	0.27	0.28	0.34	0.89
Osteoporotic patients						
6	♂	59	0.03	0.20	0.20	0.43
7	♀	65	0.02	0.05	0.20	0.27
8	♀	73	0.04	0.10	0.21	0.35
9	♂	47	0.11	0.15	0.16	0.42
10	♀	60	0.16	0.17	0.25	0.58
11	♀	81	0.04	0.14	0.15	0.33
12	♀	64	0.06	0.11	0.22	0.39
13	♂	85	0.01	0.04	0.08	0.13
14	♀	71	0.03	0.11	0.19	0.33
15	♀	77	0.15	0.10	0.12	0.37
16	♀	74	0.10	0.16	0.12	0.38
17	♀	72	0.01	0.08	0.11	0.20
18	♀	46	0.05	0.11	0.15	0.31

on filter paper disk with suction. The activity in the layer on the filter paper disk was determined in shielded sample changer with an endwindow Geiger counter (Tracerlab mod. TGC).

All counts were compared to standards prepared in an identical manner from aliquots of the administered dose of ^{45}Ca. A reagent blank was counted as background sample.

Urine samples were added to concentrated hydrochloric acid solution. Acidity was neutralized with NH_3. Calcium gluconate and ammonium oxalate were also added.

Feces were homogenized and weighed. A 10 g sample was ashed in muffle furnace at 800° C. The residue was dissolved in 10 ml of 10 % perchloric acid. Calcium was precipitated in the usual manner

All counts were corrected for self-absorption using an experimentally determined correction curve. Counting was performed on samples at infinite thickness.

It was unnecessary to correct counts for decay since the standards were always recounted together with any group of samples.

Table III ^{45}Ca in stools (% of dose)

Pat.	Sex	Age	0—72 hours
Normals			
1	o	31	35
2	♂	62	32
3	♂	76	31
4	♀	28	58
5	♀	30	29
Osteoporotic patients			
6	♂	59	52
7	♀	65	56
8	♀	75	60
9	♂	47	48
10	♀	60	41
11	♀	81	55
12	♀	64	58
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It is interesting to note the different levels of radioactivity reached by the two groups. In normal subjects, the highest value was between 2 and 2.5 % of the dose per litre of plasma. In subjects affected by osteoporosis the highest value was between 1 and 1.5 % of the dose per litre of plasma.

Table I Plasma activities (of dose per litre of plasma)

Pat.	Sex	Age	Minutes											
			5	10	15	20	25	30	40	50	60	90	120	240
Normals														
1	♂	31	–	–	+	0.35	0.70	1.00	1.80	2.00	2.15	2.60	2.61	2.40
2	♂	62	–	+	0.20	0.60	0 75	1.00	1.50	1.00	2.10	2.30	2.25	1.90
3	♂	76	–	–	+	0.20	0.50	0.70	1.20	1.60	2.00	2.15	2.25	1.95
4	♀	28	–	+	0.30	0 70	1.00	1.20	1.60	1.95	2.30	2.50	2.40	2.18
5	♀	30	–	–	+	0.20	0.60	0.90	1.50	2 10	2.30	2 40	2.35	2.10
Osteoporotic patients														
6	♂	59	–	–	–	+	0.30	0 40	0.70	1 00	1.10	1.30	1.30	1.20
7	♀	65	–	–	–	–	–	+	0 40	0.60	0.80	1.05	1 15	1.10
8	♀	75	–	–	–	–	+	0 10	0.30	0.45	0.60	0.80	0.90	0.80
9	♂	47	–	–	–	–	0.20	0 40	0.68	0.90	1 15	1.50	1.60	1 40
10	♀	60	–	–	–	–	+	0 40	0 70	1 00	1.50	1.80	1.90	1.20
11	♀	81	–	–	–	–	+	0.20	0.50	0.80	1 10	1.50	1.70	1.51
12	♀	64	–	–	–	–	+	0.20	0.50	1.00	1 10	1 40	1.36	1.25
13	♂	85	–	–	–	–	+	0.10	0.20	0.40	0.45	0.50	0.55	0.50
14	♀	71	–	–	–	–	–	+	0.40	0 70	0.90	1.00	1 10	1.00
15	♀	77	–	–	–	–	+	0 10	0.30	0.45	0.60	0.80	0.90	0.80
16	♀	74	–	–	–	–	–	+	0.20	0.50	0.65	0.90	1.00	0.85
17	♀	72	–	–	–	–	–	+	0.10	0.30	0.45	0.70	0 75	0.60
18	♀	46	–	–	–	–	+	0 10	0.40	0.70	1.00	1 70	1.85	1.60

+ = Small amounts of radioactivity

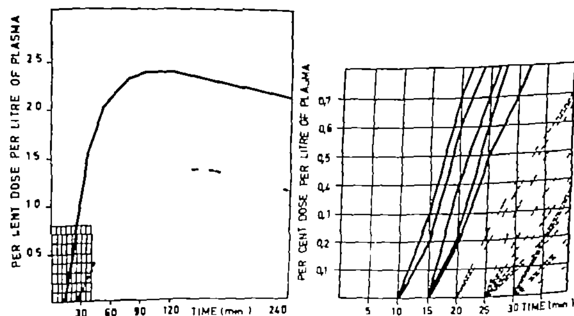


Fig. 1 Plasma activities in normals and osteoporotic patients. Left the solid line (—) represents the mean of values in normals (5 cases) the dashed line ( ) represents the mean of values in osteoporotic patients (13 cases). Right: plasma activities in the first 30 minutes.

might result from a failure of the duodenal mucosa to adjust to the low intake of calcium.

Summary

An oral dose of ^{47}Ca was administered to 15 subjects affected by senile osteoporosis and to 5 normal subjects (2 old and 3 young). In all the subjects affected by osteoporosis a defective intestinal absorption of calcium was observed.

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1. Albright F & Reifenstein E. C. The parathyroid glands and metabolic bone disease. Williams-Wilkins Co., Baltimore 1948.
2. Blau, M., Spencer, H., Swernov J & Laszlo D. Utilization and intestinal excretion of calcium in man. Science *120*: 1029 1954
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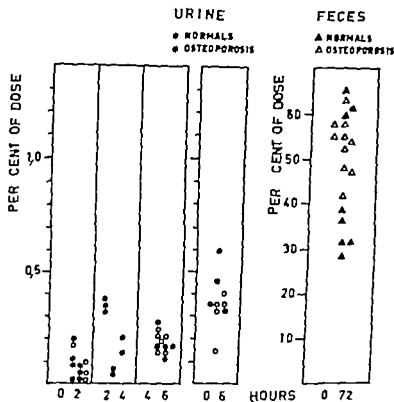


Fig 2 Excretion of ^{47}Ca in urine and feces in normals and osteoporotic patients.

The difference in the behaviour of plasma radioactivity can be summarized as follows

a) *Radioactivity appears later in the plasma*

b) *The peak radioactivity is lower even 24 hours after administration of isotope*

c) *Radioactivity is slower in reaching its peak*

The study of radioactivity in urine and feces helped in determining intestinal absorption of the isotope (tables II and III fig 2)

In subjects affected by osteoporosis urinary radioactivity increases but slightly during the three two-hour periods. The total urinary excretion of the isotope in the urine taken over a period of 6 hours was notably less than in normal subjects. In 6 hours, a normal subject eliminates in the urine from 0 80 to 1.20 % while the osteoporotic subject eliminates only from 0 15 to 0 55 % (table II fig 2)

The degree of radioactivity in the feces taken over three days was, in normal subjects, from 28 to 38 % and in osteoporotic subjects from 48 to 65 % of the dose of ^{47}Ca administered (table III fig 2)

The results of this experiment seem to resolve all doubts about the existence of a defect in the intestinal absorption of calcium in senile osteoporosis. Such a defect is accompanied by the following characteristics

a) The quantity of ^{47}Ca eliminated with feces is much greater than in the normal subject

b) The quantity of ^{47}Ca eliminated with urine is much smaller

c) ^{47}Ca appears much slowly in the blood and reaches levels of radioactivity which are definitely lower than normally

According to Lenzi and Caniggia (3) the pathogenesis of senile osteoporosis

might result from a failure of the duodenal mucosa to adjust to the low intake of calcium.

Summary

An oral dose of ^{47}Ca was administered to 18 subjects affected by senile osteoporosis and to 5 normal subjects (2 old and 3 young). In all the subjects affected by osteoporosis a defective intestinal absorption of calcium was observed.

References

1. Albright, F. & Reifenstein, E. C. The parathyroid glands and metabolic bone disease. Williams-Wilkins Co., Baltimore 1948.
2. Blau, M., Spencer, H., Swernov, J. & Laszlo, D. Utilization and intestinal excretion of calcium in man. Science *120:* 1029, 1954.
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The Elimination from Plasma of Intravenous Heparin

An Experimental Study on Dogs and Humans

By

Per Olsson Hans Lagergren and Stig Ek

Investigations on the elimination of intravenously injected heparin from the blood have been made during the past three decades by a number of authors, using different methods to determine the heparin content of blood. The results reported are essentially identical. Thus, the concentration in the blood falls rapidly after injection, and the higher the heparin dose, the more heparin is eliminated per time unit. More detailed knowledge of the disappearance of injected heparin from the blood stream is however required as a basis for further studies of the physiology of this substance. The object of the present investigation is to provide better information about the course of heparin elimination from the plasma in dogs and in human subjects, following doses of varying size. The method used for heparin determination was originally described by Blombäck et al. (5) and its usefulness for frequent determinations of the heparin concentration in plasma was later demonstrated both in dogs (20) and in man (6)

Submitted for publication November 19, 1962.

Material

The material consisted of 22 adult dogs, weighing between 12 and 20 kg, and 13 healthy subjects (6 males and 7 females) whose age ranged from 19 to 45 years (mean 27.6)

The dogs were anaesthetized by intraperitoneal injection of 10 mg/kg body weight of Nembutal® (Abbott) followed by tracheal intubation. If necessary additional Nembutal was injected intravenously during the experiments. The dogs were kept normothermic with heating pads.

Methods

Catheterization

A polyethylene catheter (PE 190) was inserted for blood sampling. In the *dogs*, it was introduced into the inferior vena cava via a peripheral vein in the hind leg. In the *humans*, it was inserted into a cubital vein by direct puncture.

Heparin administration and dosage

Commercial heparin (Vitrum Comp., Stockholm, Sweden) in 5 % solution, containing about 100 IU/mg, was used in all experiments. It was administered to the *dogs* in foreleg, and to the *human subjects* in a vein

of the arm contralateral to that in which the catheter was inserted.

In *dogs* the heparin doses were 200, 400 and 800 IU/kg body weight (6 animals in each group) and 4 800 IU/kg (4 dogs). In the *human subjects* the doses were 100 IU/kg body weight (5 persons) and 200 and 400 IU/kg (4 subjects in each group).

Blood samples

A sample for heparin determination was taken in every case before injection of heparin. In the *dogs* given 200, 400 and 800 IU samples were taken 10 min. after heparin injection in all groups and then every 20 min. up to 150 min. in the 200 IU group, every 20 min. up to 230 min. in the 400 IU group, and every 20 min. up to 270 min. in the 800 IU group. In the dogs given 4 800 IU/kg body weight, samples were taken 15 and 30 min. after injection and then every 30 min. up to 570 min.

In the *human subjects* given 100 IU/kg body weight, samples were taken every 15 min. after heparin injection up to 150 min. In the 200 and 400 IU groups, samples were taken 15 and 30 min. after injection, and then every 30 min. up to 300 and 480 min., respectively.

Heparin determinations

Each blood sample (4.5 ml) was withdrawn into a 5 ml syringe containing 0.5 ml of 3.8 sodium citrate. The sample was centrifuged within one hour at 2 000—3 000 *g* for 20 min. The plasma was pipetted off and then kept deep-frozen until it could be analyzed.

The method used for heparin determination has been described in detail elsewhere (6). The method is a thrombin titration. A constant amount of thrombin is added to the plasma to be investigated, which contains an added excess of heparin co-factor, i. e. bovine plasma. In this system, the clotting time is a function of the heparin concentration in plasma. The plasma heparin concentration is then read (in both human subjects and in dogs) against a standard curve, obtained from the different clotting times in normal human plasma, to which are added known amounts of heparin, excess of heparin co-factor and a constant amount of thrombin. When the heparin activity of the plasma to be investigated is more than about 1.0 IU/ml, it is diluted with normal human plasma to bring it into the range of the standard curve. In the present experiments the slope of the standard curves was such that heparin activities below 0.3 IU/ml of plasma could not be accurately measured. With heparin concentrations ranging from 0.48 to 12.0 IU/ml of plasma the error of the measurement was one per cent.

Statistical analyses

Statistical analyses were made in all groups except that comprising the dogs given 4 800 IU of heparin/kg body weight. In every case, the plasma heparin activity after injection of heparin fell approximately exponentially with time (table III). The logarithmic values of the heparin activities measured were therefore used in the analyses. The analyses were made during a period such that for each individual, whatever the dose group, the same number of values were taken. In the *dogs*, these periods were 130, 170 and 230 min. in the respective dose groups. The corresponding periods in the *human subjects* were 120, 270 and 360 min., respectively.

The purpose of the statistical analyses was:

1. To test whether or not the course of the heparin elimination was exponential with time.

2. To test whether the laws of heparin elimination differed with the size of the dose.

As the material resulted from a factorial experiment with the factors dose and time, each with three or more levels, the following statistical analysis was made so as to give as much information as possible from the data at hand. The analysis is a combination of analysis of regression and analysis of variance with due account of all individual degrees of freedom.

Symbols

C = dogs, T_1, T_2, T_3 and T_4 denote orthogonal polynomials in the time of 1st, 2nd, 3rd and 4th degree respectively (11). D_1 and D_2 denote orthogonal polynomials in dose of 1st and 2nd degree, respectively.

Example: $(C \times T_1)$ = interaction between individual dogs and the 1st degree polynomial

in time (measure of the variation between T in dogs given the same dose)

x = almost significant ($0.05 > p > 0.01$)

xx = significant ($0.01 > p > 0.001$)

xxx = highly significant ($p < 0.001$)

Calculations

A series of orthogonal polynomials in time up to the 4th degree was calculated for each individual. These orthogonal functions mean, 1st, 2nd, 3rd and, in some cases 4th, degree polynomials characterize most of the variations in the values for one individual. The residual variation is attributed to uncontrollable fluctuations. The semilogarithmic elimination curve for one individual is thus characterized by the mean of all measured heparin values, by the slope (1st degree polynomial) by the curvature (2nd degree polynomial) by the S-shape (3rd degree polynomial) and, in some individuals, also by 4th degree curvature (4th degree polynomial). An analysis of variance (22) was made with the orthogonal polynomials obtained by the calculations in subgroups (e. g. dogs given 800 IU heparin/kg (table I)) and in groups (e. g. all dogs (table II))

Dogs

The analysis of variance for the dogs given 800 IU/kg is shown in table I The mean square for the difference between the means of the individual dogs and the mean square for the interaction between this term and the polynomials in time was tested against the mean square for the residual ariation The differences between the mean values for heparin content in the individual dogs (C) were highly significant, as were the differ ences between the slopes of the heparin elimination curves in the individual animals (C × T) The interactions between C and polynomials of higher degree (C T) were not significant, e. no difference could be demonstrated between the curvatures of the heparin elimination curve in the individual animals.

In order to assess the shape of the semilogarithmic heparin elimination curve generally valid for dogs given heparin dose of 800 IU/kg, the term T was tested against the term C × T and T T and T against C × T T was highly significant, indicating that the heparin activity in plasma decreases with time (which is obvious) T T and T were almost significant, thus indicating that the semilogarithmic elimination curve generally valid in this dose group may have curvatures of the 2nd, 3rd and 4th degree.

The esults of the corresponding calculations for the dogs given heparin doses of 400 and 200 IU/kg body weight, respectively are essentially the same as in the preceding group, except that the semilogarithmic elimination curves for the doses 400 and 200 IU/kg may

Table I. Analysis of variance of values obtained by calculation of orthogonal polynomials dogs, heparin dose 800 IU/kg BW Symbols, see text

Source of variation	Significance
C	
T	
C × T	
T	
T	
T	
C × T	—

Table II Analysis of variance of values obtained by calculation of orthogonal polynomials: dogs, heparin doses 800 400 and 200 IU/kg BW Symbols see text

Source of variation	Significance
D_1	
D_2	—
C	
T	
D_1 × T	—
D × T	—
C T	
T	
D × T	—
D_1 T	—
T	—
D_1 × T	—
D T	—
C T	

of the arm contralateral to that in which the catheter was inserted

In *dogs* the heparin doses were 200, 400 and 800 IU/kg body weight (6 animals in each group) and 4 800 IU/kg (4 dogs). In the *human subjects* the doses were 100 IU/kg body weight (5 persons) and 200 and 400 IU/kg (4 subjects in each group)

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A sample for heparin determination was taken in every case before injection of heparin. In the *dogs* given 200, 400 and 800 IU samples were taken 10 min. after heparin injection in all groups and then every 20 min. up to 150 min in the 200 IU group, every 20 min. up to 230 min. in the 400 IU group, and every 20 min. up to 270 min in the 800 IU group. In the dogs given 4 800 IU/kg body weight samples were taken 15 and 30 min after injection, and then every 30 min. up to 570 min.

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Statistical analyses

Statistical analyses were made in all groups except that comprising the dogs given 4 800 IU of heparin/kg body weight. In every case, the plasma heparin activity after injection of heparin fell approximately exponentially with time (table III) The logarithmic values of the heparin activities measured were therefore used in the analyses. The analyses were made during a period such that for each individual, whatever the dose group, *the same* number of values were taken. In the *dogs*, these periods were 130, 170 and 230 min. in the respective dose groups. The corresponding periods in the *human subjects* were 120, 270 and 360 min., respectively

The purpose of the statistical analyses was

1 To test whether or not the course of the heparin elimination was exponential with time.

2 To test whether the laws of heparin elimination differed with the size of the dose

As the material resulted from a factorial experiment with the factors dose and time, each with three or more levels, the following statistical analysis was made so as to give as much information as possible from the data at hand. The analysis is a combination of analysis of regression and analysis of variance with due account of all individual degrees of freedom.

Symbols

C = dogs, T_1, T_2, T_3 and T_4 denote orthogonal polynomials in the time of 1st, 2nd, 3rd and 4th degree, respectively (11) D_1 and D_2 denote orthogonal polynomials in dose of 1st and 2nd degree, respectively

Example ($C \times T_1$) = interaction between individual dogs and the 1st degree polynomial

Table III A. Plasma heparin activity after injection of heparin in human subjects

Time after injec. (min)	Heparin IU/ml plasma					Time after injec. (min)	Heparin IU/ml plasma							
	Dose 100 IU/kg BW						Dose 200 IU/kg BW				Dose 400 IU/kg BW			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1.20	2.0	1.58	1.29	1.23	15	2.88	2.92	3.16	3.40	6.82	7.50	7.74	7.52
30	1.00	1.65	1.18	0.98	1.13	30	2.52	2.43	2.64	2.80	5.60	7.0	6.93	5.88
45	0.77	1.50	0.98	0.82	0.96	60	1.98	1.83	2.16	2.28	4.28	6.0	5.13	—
60	0.71	1.12	0.84	0.68	0.69	90	1.62	1.48	1.74	1.83	3.57	5.22	4.48	4.08
75	0.54	1.0	0.64	0.53	0.60	120	1.22	1.18	1.46	1.34	3.18	4.44	4.20	3.75
90	0.49	—	0.54	0.47	0.62	150	0.93	1.04	1.14	1.23	2.82	3.83	3.51	3.48
105	0.41	0.72	0.39	0.37	0.52	180	0.80	0.76	0.83	1.19	2.48	3.24	2.93	2.90
120	0.35	0.67	0.30	0.31	0.48	210	0.61	0.63	0.64	0.87	2.09	3.08	2.80	2.40
135		0.55			0.39	240	0.42	0.52	0.54	0.77	1.88	2.76	2.48	1.88
150					0.31	270	0.39	0.49	0.43	0.35	1.58	2.25	2.27	1.80
						300		0.42			1.32	1.98	1.93	1.71
						330					1.11	1.83	1.87	1.50
						360					0.93	1.56	1.52	1.24
						390					0.79		1.26	
						420					0.73			
						450					0.62			
						480					0.55			

injection. In the dogs, however some activity was found in every case and ranged from 0.15 to 0.60 IU/ml (mean 0.30 ± 0.006 IU/ml)

The total amount of heparin eliminated from the plasma per time unit was always greater initially in both dogs and man and gradually fell with decreasing plasma heparin activity. A typical curve from a human subject is shown in fig 1. When plotted on the semilogarithmic scale, the plasma heparin activity decreased approximately exponentially with time in all experiments, except in the dogs given the highest dose (4 800 IU/kg). The statistical calculation disclosed however that the course of heparin elimination was strictly exponential with time only in the human group given a heparin injection of 100 IU/kg of body weight. In the human groups given 200 and 400 IU/kg the elimination was exponential *only* from 30 minutes after injection. Before 30 minutes, there was a tendency *to a* higher relative elimination rate than after. The most striking finding with respect to heparin elimination in the human was, however that the relative elimination rate during the exponential part of elimination in the different groups decreased with an increasing dose. The average semilogarithmic elimination curves for th different doses in the human subject are shown in fig. 2.

In the dogs given a heparin injection of 200 400 and 800 IU/kg the average semilogarithmic elimination curves during the first 150 minutes after injection had parallel curvatures of the 2nd degree. Thus, during this period the relative

have curvatures of the 2nd and 3rd and of the 2nd degree respectively.

To determine whether differences were present between the relevant dose groups, and also to extract as much information as possible from the material with respect to the course of heparin elimination, calculations were made on values from all the dogs during the first 130 min. after injection, i. e. during the period when all had the same number of values (table II). D_1 denotes the difference between the smallest and the largest dose and D_2 is a measure of the "non-linear dose effect", which would be significant if the result for the middle dose did not lie halfway between the other two. D_1 was found to be highly significant, whereas D_2 was not significant. As before, C and $C \times T_1$ were tested against the mean square of the residual variation and, as before, the difference between dogs given the same heparin dose was highly significant with respect to both the mean heparin activity in plasma (C) and the slope of the regression line ($C \times T_1$). In this material of three-fold size there were — contrary to the findings when each group was tested separately — highly significant differences between the curvatures of the heparin elimination curve in the individual animals given the same dose ($C \times T_2$, T_3).

The mean course of the heparin elimination curve in all the dogs was obtained by testing T_1, T_2 and T_3 against $C \times T_1$ and $C \times T_{2,3}$ respectively. T_1 and T_2 were highly significant, thereby proving that the semilogarithmic heparin elimination curve had a curvature of the 2nd degree in addition to the obvious slope. To ascertain whether the course of heparin elimination differed with the dose, the terms $D_1 \times T_1$ and $D_2 \times T_1$ were tested against $C \times T_1$. This implies a comparison between the difference in the slope of the semilogarithmic elimination curves for the three doses and the same differences within the respective dose groups. $D_1 \times T_2$, $D_1 \times T_3$, $D_2 \times T_2$ and $D_2 \times T_3$ were tested in the same way against $C \times T_{2,3}$ to compare the differences in the curvatures. None of these effects were significant. Thus, there was no difference in the course of heparin elimination among the three dose groups, i. e., the semilogarithmic elimination curves ran parallel during the first 130 min. after the injection.

Human subjects

When calculating the orthogonal polynomials in time, the value 15 min. after injection in the groups given heparin doses of 200 and 400 IU/kg body weight was omitted, since this type of analysis requires the periods of time between the terms to be equal. An analysis of variance was made in the different dose groups, as in the dogs. The results showed that the heparin concentration in all the dose groups decreased exponentially with time (T_1 highly significant, T_2 and T_3 not significant). As with the dogs, there were highly significant variations between individuals given the same heparin dose.

Calculations were made on all the values up to 120 min. after injection in the whole material, as in the dogs. To obtain the necessary symmetry, one individual in the 100 IU/kg group was omitted, as well as the values at 15, 45, 75 and 105 min. in this group. As before, heparin elimination followed a linear exponential course. The mean course of the elimination curves was, however, found to differ in the three dose groups ($D_1 \times T_1$ highly significant), the variation in the slope being approximately inversely proportional to the logarithm of the dose ($D_2 \times T_1$ not significant).

In order to test whether the values at 15 min. in the individuals given 200 and 400 IU/kg deviated from the straight regression line, the following calculation was performed. The difference between the observed value and the regression value at the time 15 min. after heparin injection was computed in each individual. These 12 values (the values from all groups) were subjected to an analysis of variance; the mean square of the error was 1 195. The mean difference between the eight values in groups with 200 and 400 IU/kg was 35.9 and the s. d. 12.2. The corresponding t value was 2.94 (df 10), which implies that these values had a strong tendency to deviate from the straight regression line.

Results

The values for plasma heparin activity obtained in the different experiments are seen in table III. In the human subjects, heparin activity was never present before

Heparin IU/ml plasma						Time after injec. (min)	Heparin IU/ml plasma			
Dose 800 IU/kg BW							Dose 4 800 IU/kg BW			
0.29	0.60	0.15	0.40	0.29	0.35		0.31	0.35	0.18	0.31
8.16	8.14	9.86	10 40	9.60	—	15	42.70	45.40	37.50	44.00
6.90	6.50	6.12	7.70	7.00	7.00	30	31.50	28.00	28.20	31 60
4.28	4 92	—	6.56	5.62	5.50	60	23.60	18.90	17.50	21.90
3 60	4.32	4.57	6.02	4.80	4.60	90	20.40	13.75	13.63	15.40
2.56	3.40	3.57	5.03	3.63	4.10	120	11 75	11.20	11 76	13.10
2.10	2.90	3 00	4.56	3.30	3.76	150	9.00	10.90	9.85	12.56
1 95	2.68	2.55	4.30	2.90	2.75	180	7.55	9 82	8.05	9.20
1.50	2.13	2.00	3 60	2.50	2.40	210	7.00	8.58	6.12	8.70
1.10	2.00	1.50	2.80	1.86	1.92	240	5.90	6.57	5.18	7.75
0.71	1.80	1.38	2.60	—	1.64	270	5.45	6.00	4.44	6.12
0.66	1.50	0.96	2.30	1.31	1 47	300	4.55	4.56	3.52	3.36
0.60	1.18	1.06	1 70	1.07	1 14	330	3.90	4.13	2.58	4.69
	0.79			0.95		360	3.24	3.42	2.35	4.50
	0.72					390	2.28	3.02		
						420	—	—		
						450	1.60	2.50		
						480	1.52	2.20		
						510	1.32	2.00		
						540	1 16	1.60		
						570	1.02	1 46		

the k value (angle coefficient) of the calculated straight regression lines (table IV) The mean time for 50 per cent decrease n the human subjects given 100 IU/kg was 56 ± 3.5 minutes, the corresponding values in the 200 IU/kg and 400 IU/kg groups being 96 ± 5 1 and 152 ± 5 0 minutes respectively In the dogs given a heparin njection of 200 400 or 800 IU/kg of body weight the mean time for 50 per cent decrease in heparin activity was 66 ± 2.5 minutes when the determination was limited to the first 130 minutes. When t was measured for the whole investigation period in the 400 and 800 IU/kg groups (170 and 230 minutes, respectively after injection) the value became somewhat higher in the 800 IU/kg group. In the dogs given a heparin dose of 4 800 IU/kg regression lines were calculated before and after the break at 120 minutes. The approximate mean time for 50 per cent decrease during the first 120 minutes was 60 ± 1.9 minutes. In the period after the break, the corresponding mean value was 150 ± 11 4 minutes.

A theoretical value for the heparin activity immediately after heparin injection was extrapolated from the straight regression lines (table V) In the human subjects the averages were 1 7 IU/ml of plasma in the 100 IU/kg group, 3.2 IU/ml in the 200 IU/kg group and 6.7 IU/ml in the 400 IU/kg group The increase in initial heparin activity was thus line-

Table III B Plasma heparin activity after injection of heparin in dogs

Time after injec. (min)	Heparin IU/ml plasma											
	Dose 200 IU/kg BW						Dose 400 IU/kg BW					
0	0.30	0.35	0.35	0.29	0.31	0.15	0.26	0.40	0.27	0.36	0.22	0.15
10	3.06	2.93	2.82	3.75	2.80	2.55	5.88	5.70	5.60	6.36	5.60	4.90
30	2.00	2.19	2.07	2.46	2.10	—	3.80	4.00	3.95	4.23	3.80	3.66
50	1.77	1.70	1.65	1.74	1.75	1.86	3.08	3.20	2.84	3.44	3.28	2.80
70	1.41	1.47	1.33	1.30	1.40	1.50	2.43	2.58	2.31	2.64	2.72	2.40
90	1.20	1.38	1.02	0.91	1.08	1.20	2.13	2.40	1.93	1.95	2.28	1.98
110	1.06	1.18	0.80	0.85	0.92	0.94	1.64	2.00	1.51	1.60	1.83	1.82
130	—	0.80	0.77	0.77	0.72	0.74	1.50	1.65	1.40	1.52	1.46	1.50
150				0.61		0.66	1.40	1.00	1.20	0.92	1.42	1.20
170				0			1.13	0.91	0.80	0.76	1.18	1.06
190								0.79				0.86
210								—				0.71
230								0.63				
250												
270												

elimination rates of the injected heparin were the same in the three groups. In the dogs given 400 and 800 IU/kg a tendency to a still more complicated course of heparin elimination occurred after 130 minutes with appearance of curvatures of the 3rd and 3rd and 4th degree, respectively In the dogs given 4 800 IU/kg the average semilogarithmic curve — plotted from the mean values of heparin concentration — deviated markedly from a straight line i. e it had a steep slope during the first 120 minutes, followed by a considerably less steep slope It therefore differed completely from corresponding curves after smaller doses. The average semilogarithmic elimination curves for the different dose groups in the dogs are presented in fig 3

It is evident from figs. 2 and 3 that — except for the dogs given 4 800 IU/kg — the heparin elimination in both human and animal groups could be represented with fairly good approximation by a straight line on the semilogarithmic scale. Since the statistical analysis disclosed that the course of heparin elimination differed significantly in all individuals, it was justifiable to calculate the relative elimination rate and the heparin activity immediately after injection in each case.

The relative elimination rate was measured as the time for 50 per cent decrease in heparin activity obtained from

Heparin IU/ml plasma						Time after injec. (min)	Heparin IU/ml plasma			
Dose 800 IU/kg BW							Dose 4 800 IU/kg BW			
0.29	0.60	0.15	0.40	0.29	0.35		0.31	0.33	0.18	0.31
8.16	8.14	9.86	10.40	9.60	—	15	42.70	43.40	37.50	44.00
6.90	8.50	6.12	7.70	7.00	7.00	30	31.50	28.00	28.20	31.60
4.20	4.92	—	6.56	5.62	5.50	60	23.60	16.90	17.50	21.90
3.60	4.32	4.37	6.02	4.80	4.60	90	20.40	13.75	13.65	13.40
2.56	3.40	5.37	5.03	3.63	4.10	120	11.75	11.20	11.76	13.10
2.10	2.90	3.00	4.56	3.30	3.76	150	9.00	10.90	9.80	12.56
1.95	2.68	2.55	4.50	2.90	2.73	180	7.55	9.02	8.05	9.20
1.50	2.13	2.00	3.60	2.30	2.40	210	7.00	8.38	6.12	8.70
1.10	2.00	1.50	2.80	1.86	1.52	240	5.90	6.57	5.18	7.75
0.71	1.80	1.38	2.60	—	1.64	270	5.45	6.00	4.44	6.12
0.66	1.30	0.96	2.30	1.31	1.47	300	4.55	4.56	3.52	5.96
0.60	1.18	1.06	1.70	1.07	1.14	330	3.90	4.13	2.58	4.69
	0.79			0.93		360	3.24	3.42	2.33	4.50
	0.72					390	2.28	3.02		
						420	—	—		
						450	1.60	2.50		
						480	1.52	2.20		
						510	1.32	2.00		
						540	1.16	1.69		
						570	1.02	1.44		

the *b* value (angle coefficient) of the calculated straight regression lines (table IV). The mean time for 50 per cent decrease in the human subjects given 100 IU/kg was 56 ± 3.5 minutes, the corresponding values in the 200 IU/kg and 400 IU/kg groups being 96 ± 3.1 and 152 ± 3.0 minutes respectively. *In the* dogs given a heparin injection of 200, 400 or 800 IU/kg of body weight, the mean time for 50 per cent decrease in heparin activity was 66 ± 2.5 minutes when the determination was limited to the first 130 minutes. When it was measured for the whole investigation period in the 400 and 800 IU/kg groups (170 and 230 minutes, respectively after *injection*) the value became somewhat higher in the 800 IU/kg *group*. In the dogs given a heparin dose of 4 800 IU/kg regression lines were calculated before and after the break at 120 minutes. The approximate mean time for 50 per cent decrease during the first 120 minutes was 60 ± 1.9 minutes. In the period after the break, the corresponding mean value was *130 ± 11.4 minutes*.

A theoretical value for the heparin activity immediately after heparin injection was extrapolated from the straight regression lines (table V). In the human subjects the averages were 1.7 IU/ml of plasma in the 100 IU/kg group, 3.2 IU/ml in the 200 IU/kg group and 6.7 IU/ml in the 400 IU/kg group. The increase in initial heparin activity was thus line

Table III B. Plasma heparin activity after injection of heparin in dogs

Time after injec. (min)	Heparin IU/ml plasma											
	Dose 200 IU/kg BW						Dose 400 IU/kg BW					
0	0.30	0.35	0.35	0.29	0.31	0.15	0.26	0.40	0.27	0.36	0.22	0.15
10	3.06	2.93	2.82	3.75	2.80	2.55	5.88	5.70	5.60	6.36	5.60	4.90
30	2.00	2.19	2.07	2.46	2.10	—	3.80	4.00	3.95	4.23	3.80	3.66
50	1.77	1.70	1.65	1.74	1.75	1.86	3.08	3.20	2.84	3.44	3.28	2.80
70	1.41	1.47	1.33	1.30	1.40	1.50	2.43	2.58	2.31	2.64	2.72	2.40
90	1.20	1.38	1.02	0.91	1.08	1.20	2.13	2.40	1.95	1.95	2.28	1.98
110	1.06	1.18	0.80	0.85	0.92	0.94	1.64	2.00	1.31	1.60	1.83	1.82
130	—	0.80	0.77	0.77	0.72	0.74	1.50	1.65	1.40	1.32	1.46	1.50
150				0.61		0.66	1.40	1.00	1.20	0.92	1.42	1.20
170				0			1.13	0.91	0.80	0.76	1.18	1.06
190								0.79				0.86
210								—				0.71
230								0.63				
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The relative elimination rate was measured as the time for 50 per cent decrease in heparin activity obtained from

Table IV Variations in time for 50 % decrease in heparin activity of plasma after i.v. injection of heparin in human subjects (A) and dog (B)

	Heparin dose (IU/kg BW)	Period after injec. (min)	Time for 50 % decrease in heparin activity (min)						Mean
A	100	30–120	61	64	46	52	63		56 ± 3.5
	200	30–120	86	108	101	89			96 ± 5.1
	400	30–120	142	154	166	151			152 ± 5.0
B	200	10–130	68	72	62	51	62	56	
	400	10–130	61	72	60	54	66	73	66 ± 2.5
	800	10–130	54	62	66	97	70	77	
	400	10–170	73	63	63	54	74	75	66 ± 3.4
	800	10–230	70	84	68	54	73	77	74 ± 4.0
	4 800	10–120	62	56	65	61			60 ± 1.9
		150–360	151	122	100	143			150 ± 11.4

$T/2 = \frac{\log 2}{b}$ where b = angle coefficient of regression line.

Table V Theoretical plasma heparin activity immediately after heparin injection, obtained by extrapolation of straight regression lines in human subjects (A) and dog (B)

	Heparin dose (IU/kg BW)	Theoretical initial heparin activity IU/ml plasma						Mean	Increase in heparin activity IU/ml plasma
A	100	1.3	2.3	1.9	1.5	1.3		1.7 ± 0.2	1.7 ± 0.2
	200	3.2	3.4	2.8	3.5			3.2 ± 0.2	3.2 ± 0.2
	400	5.8	7.7	6.8	6.5			6.7 ± 0.4	6.7 ± 0.4
B	200	3.0	3.0	3.0	3.7	3.0	3.0	3.1 ± 0.1	2.8 ± 0.1
	400	5.3	6.0	5.3	6.6	5.4	4.8	5.6 ± 0.3	5.3 ± 0.3
	800	6.5	7.8	8.9	12.3	9.6	9.2	9.1 ± 0.8	8.8 ± 0.8
	4 800	49.1	44.7	38.2	46.9			44.7 ± 2.4	44.4 ± 2.5

Obtained by subtraction of the mean preinjection value (0.3 IU/ml) from the theoretical heparin activity immediately after injection.

practical grounds, the heparin determinations in canine plasma were also made against a standard curve with human plasma. The finding of some heparin activity in canine plasma before injection of heparin — as compared to its absence in man — is compatible with the fact that higher heparin activity can be recovered from the organs of the dog than from human organs (3). It might, however also have been due to a lack of congruence between canine and human plasma in the test system used.

Elimination of the heparin injected per time unit was greatest immediately after injection, and decreased gradually with falling activity in the plasma. This is in conformity with the findings in experiments on the dog made by Jaques (12) Monkhouse and Jaques (19) Da-

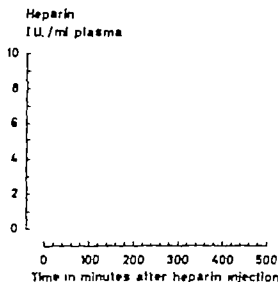


Fig 1 Elimination of heparin from plasma in a normal human subject, linear scale Dose 200 IU/kg body weight.

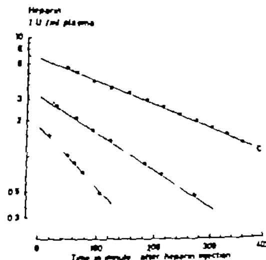


Fig 2 Elimination of heparin from plasma in humans, at three different heparin doses semi-logarithmic scale A = 50 IU/kg body weight, B = 100 IU/kg body weight, C = 200 IU/kg body weight.

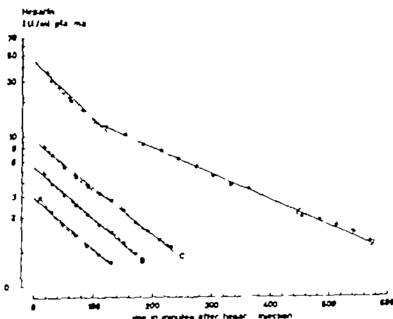


Fig 3. Elimination of heparin from plasma in dogs at four different heparin doses, semi-logarithmic scale. A = 100 IU/kg body weight, B = 200 IU/kg body weight C = 400 IU/kg body weight, D = 2 400 IU/kg body weight.

arly proportional to the size of the dose calculated from the regression line. Such a proportionality was also considered to apply in the dogs, even if there was a tendency to lower values than expected with rising dose, especially with a dose of 4 800 IU/kg

Discussion

In the present experiments, the heparin activity of the plasma measured before injection of heparin was always zero in the human subjects. This was expected since the heparin determination in plasma is made as a comparison with the conditions in normal human plasma. On

the human organism to eliminate heparin thus seems to be dependent on the size of the dose. In the dogs, this phenomenon of dose dependence appeared after a dose of 4 800 IU/kg

Many different processes have been shown to partake in removal of exogenous heparin from the blood stream. Von Kaulla and Pratt (15) demonstrated the presence of heparin in the extravascular space after intravenous injection of heparin in the dog Piper (21) Eiber and Danishefsky (9) and Loomis (16) showed that after injection heparin was rapidly deposited in various organs, chiefly the liver the spleen and the lungs. Metabolic breakdown has been observed by Jaques and Keen-Szanto (13) Jaques et al. (14) Danishefsky and Eiber and Eiber et al. Excretion through the kidneys has been reported by many workers (2, 7 8 10 14 17 18, 21 23)

The contribution of each of these processes to the total capacity of the organism to eliminate heparin has not been elucidated. Obviously it is also impossible to decide, on the basis of the present experiments, which process or processes may be responsible for the fact that, in the dog, a dose of 4 800 IU/kg is initially eliminated so much faster in relative terms, than a dose of e. g 800 IU/kg Nor can it be determined which process or processes can comprise the dose dependence during elimination.

Nevertheless comparisons can be made with earlier observations. Jaques et al. found in experiments on the dog, that the part of the injected heparin that was not recovered in the urine must have been taken up by some system whose capacity was, however limited Danishefsky and Eiber as well as Eiber et al. injected radioactive heparin into dogs and human subjects, in doses corresponding to those used in the present study They found that the amount of radioactivity excreted in the urine during 24—48 hours after injection comprised — particularly in man — a successively smaller proportion of the dose with an increase in its size. That part of the excreted radioactivity which represented unchanged heparin showed a relative increase with a rise in the dose whereas the part consisting of metabolites of heparin showed a relative decrease. The authors interpreted their results to imply that the organism has a limited ability to metabolize heparin. This may be an explanation of the decrease in the relative rate of elimination with increasing doses of heparin in man. It might also explain why the relative rate of elimination becomes slower in the later part of the course in dogs given an extremely large heparin dose.

Summary

Heparin was injected intravenously in varying doses into dogs and healthy human subjects. The heparin activity of the plasma was determined at fixed intervals after injection by a thrombin titration method.

In *dogs* the elimination of heparin from plasma is found to be approximately exponential with time following doses of 200 to 800 IU/kg of body weight. With these doses, the time for 50 per cent decrease in heparin activity is found to be about 66 minutes. The elimination course of a dose of 4 800 IU/kg has two phases, i. e. an initial one with a high relative rate, and a later one with a low relative rate of elimination.

In *human subjects* the heparin is found to be eliminated from plasma exponentially with time after a dose of 100 IU/kg of body weight, and approximately expo-

nishefsky and Eiber (8) Andersen et al (1) and Olsson et al (20) as well as with the observations made in man by Blombäck et al (6) and Eiber et al (10)

Andersen et al and Olsson et al suggested that heparin injected into the dog is eliminated exponentially with time The present experiments nevertheless *showed a small but highly significant* deviation from such a course. The course of heparin elimination in the dog is in fact always too complicated to allow it to be depicted as a straight line on the *semilogarithmic scale. After doses ranging* from 200 to 800 IU/kg the mean course was however the same during comparable times after injection At these doses the elimination could be regarded from *the practical aspect as taking place ex*ponentially Moreover the fact that the time for 50 per cent decrease in heparin activity was the same (approximately 66 minutes) at different doses implies that at each time after injection of heparin the heparin activity of the plasma is in linear proportion to the size of the dose The period during which the heparin activity is in excess of a certain value does not therefore increase in linear proportion to the size of the dose. At each doubling of the dose the duration *of the heparin activity above that certain* value increases only with the half life time of injected heparin

The conditions differ with the extremely large dose of 4 800 IU/kg of body weight During the first phase of the bisected course of elimination — when the main part of the injected heparin was eliminated — the plasma heparin activity was lower than expected on each measurement. This might have been due to the relative rate of elimination immediately after injection being high as compared to that after the lower doses.

The time of elimination of the dose as a whole must however be influenced by the later relatively slower phase.

In human subjects given a heparin dose of 100 IU/kg the course of its elimination was exponential with time. After doses of 200 and 400 IU/kg there was, however an indication of the course being divided into two phases. *The later* phase could with good approximation, be regarded as reflecting the whole course of elimination The time for 50 per cent decrease in heparin activity was *not the same at the different doses, but* increased approximately in proportion to the logarithm of the dose. This has the practical consequence that, with clinically applicable doses, the period for which *plasma heparin activity persists* above e. g 0.5 IU/ml increases more than in proportion to the increase in dose. Thus, after a dose of 100 IU/kg about 100 minutes are required for the heparin concentration to have fallen to 0.5 IU/ml of plasma whereas after a dose of 200 IU/kg it takes about 250 minutes for this value to be reached *After a dose* of 400 IU/kg the corresponding mean value would be about 600 minutes if — as indicated by the only case followed thus far (fig 1) — the course of elimi*nation is also exponential* in this group down to 6.5 IU/ml of plasma.

Despite the decrease in the relative rate of elimination with rising *doses of* heparin in the human subjects, the absolute quantity of heparin eliminated during the same periods of time after injection is greater after a large dose than after a smaller *one* When after doses of varying size the heparin activity of the plasma has fallen to the same value, the quantity of heparin eliminated per time unit is, however less after a large dose than after a smaller one. The ability of

the human organism to eliminate heparin thus seems to be dependent on the size of the dose. In the dogs, this phenomenon of dose dependence appeared after a dose of 4 800 IU/kg

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In *human subjects* the heparin is found to be eliminated from plasma exponentially with time after a dose of 100 IU/kg of body weight, and approximately expo-

nentially with time after doses of 200 and 400 IU/kg The time for a 50 per cent decrease in heparin activity increases with the amount of heparin injected Thus, with a dose of 100 IU/kg it is found to be on an average 56 minutes, the corresponding average times after doses of 200 and 400 IU/kg being 96 and 152 minutes, respectively

Acknowledgement

Supported by grants from the Karolinska Institutet, the Swedish Association for Heart *and Lung Diseases and Torsten och Ragnar* Söderbergs Foundation for Medical Research.

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Studies in Neurocirculatory Asthenia

III. On the Etiology and Pathogenesis of Signs in the Work Test and Orthostatic Test

By

Maj Levander-Lindgren*

In a preceding paper (27) a report was given of the symptomatology and the findings in the work test and orthostatic test in 150 patients with neurocirculatory asthenia (NCA). There was observed a high frequency of marked orthostatic reactions, of low physical working capacity (PWC) and of sympathicotonic ECG changes with depression of the S-T segment and flattening of the T wave at rest and after work. In a subsequent paper (28) a statistical analysis was made of the correlations between symptoms and signs and the influence of immobilization and body index. This paper will give a brief review of the etiology and discuss the pathogenesis of the abnormal findings in the work test and orthostatic test.

Etiologic factors

Most authors agree that NCA is a neurosis, akin to anxiety neurosis (1, 2, 3, 4, 11, 14, 21, 40, 41, 42, 43, 44, 45, 46). It is not surprising that one commonly finds hereditary constitutional factors (10, 12, 23, 40, 44, 45) as well as psychic stress and personal experience of heart disease in these patients (3, 5, 7). Somatic diseases such as infections and operations may precipitate NCA, especially the acute type (22, 33, 37).

In the present series of NCA strong psychological traumata had occurred in 40 patients. Many of these patients had grown up in broken homes, and experienced apathy and insecurity. Twenty-nine patients had some infection or operative procedure before the onset of the condition. NCA was preceded by organic heart disease or arrhythmia in 19 patients, and in 6 cases iatrogeneses was observed. Overwork with psychical as well as physical strain was considered the predisposing factor in 19 patients. Often several predisposing factors were present at the same time. The possibility of cerebral lesions

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nentially with time after doses of 200 and 400 IU/kg The time for a 50 per cent decrease in heparin activity increases with the amount of heparin injected Thus, with a dose of 100 IU/kg it is found to be on an average 56 minutes, the corresponding average times after doses of 200 and 400 IU/kg being 96 and 152 minutes, respectively

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From the Department of Internal Medicine, Karolinska sjukhuset Stockholm, Sweden

Studies in Neurocirculatory Asthenia

III. On the Etiology and Pathogenesis of Signs in the Work Test and Orthostatic Test

By

Maj Levander Lindgren[1]

In a preceding paper (27) a report was given of the symptomatology and the findings in the work test and orthostatic test in 190 patients with neurocirculatory asthenia (NCA). There was observed a high frequency of marked orthostatic reactions, of low physical working capacity (PWC) and of sympathicotonic ECG changes with depression of the S-T segment and flattening of the T wave at rest and after work. In a subsequent paper (28) a statistical analysis was made of the correlations between symptoms and signs and the influence of immobilization and body index. This paper will give a brief review of the etiology and discuss the pathogenesis of the abnormal findings in the work test and orthostatic test.

Etiologic factors

Most authors agree that NCA is a neurosis, akin to anxiety neurosis (1 2, 3 4 11 14 21 40, 41 42, 43 44 45 46). It is not surprising that one commonly finds hereditary constitutional factors (10 12, 23 40 44 45) as well as psychic stress and personal experience of heart disease in these patients (3 5 7). Somatic diseases such as infections and operations may precipitate NCA, especially the acute type (22, 33 37).

In the present series of NCA strong psychological traumata had occurred in 40 patients. Many of these patients had grown up in broken homes, and experienced apathy and insecurity. Twenty-nine patients had some infection or operative procedure before the onset of the condition. NCA was preceded by organic heart disease or arrhythmia in 19 patients, and in 6 cases iatrogeneses was observed. Overwork with psychical as well as physical strain was considered the predisposing factor in 19 patients. Often several predisposing factors were present at the same time. The possibility of cerebral lesions

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nentially with time after doses of 200 and 400 IU/kg. The time for a 50 per cent decrease in heparin activity increases with the amount of heparin injected. Thus, with a dose of 100 IU/kg it is found to be on an average 56 minutes, the corresponding average times after doses of 200 and 400 IU/kg being 96 and 152 minutes respectively.

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III. On the Etiology and Pathogenesis of Signs in the Work Test and Orthostatic Test

By

Maj Levander Lindgren

In preceding paper (27) a report was given of the symptomatology and the findings in the work test and orthostatic test in 130 patients with neurocirculatory asthenia (NCA). There was observed a high frequency of marked orthostatic reactions, of low physical working capacity (PWC) and of sympathicotonic ECG changes with depression of the S-T segment and flattening of the T wave at rest and after work. In a subsequent paper (28) a statistical analysis was made of the correlations between symptoms and signs and the influence of immobilization and body index. This paper will give a brief review of the etiology and discuss the pathogenesis of the abnormal findings in the work test and orthostatic test.

Etiologic factors

Most authors agree that NCA is a neurosis, akin to anxiety neurosis (1, 2, 3, 4, 11, 14, 21, 40, 41, 42, 43, 44, 45, 46). It is not surprising that one commonly finds hereditary constitutional factors (10, 12, 23, 40, 44, 45) as well as psychic stress and personal experience of heart disease in these patients (3, 5, 7). Somatic diseases such as infections and operations may precipitate NCA, especially the acute type (22, 33, 37).

In the present series of NCA strong psychological traumata had occurred in 40 patients. Many of these patients had grown up in broken homes, and experienced apathy and insecurity. Twenty nine patients had some infection or operative procedure before the onset of the condition. NCA was preceded by organic heart disease or arrhythmia in 19 patients, and in 6 cases iatrogenesis was observed. Overwork with psychical as well as physical strain was considered the predisposing factor in 19 patients. Often several predisposing factors were present at the same time. The possibility of cerebral lesions

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Table I Influence of etiologic factors on the symptomatology and signs in the work test. (For definition of groups see text)

Group	Total no. of patients	Dominating psychic symptoms		Precordial pain		Palpitations		Normal findings in the work test	
		No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%
Psychogenic	35	17	49	32	91	25	71	9	26
Convalescent	23	5	22	17	74	19	83	2	9

was considered in 2 patients in one of them after head injury and in the other after encephalitis. In 17 cases the etiology was unknown The occurrence of the condition in three sisters and in a mother and her daughter favours hereditary constitutional factors.

Influence of etiological factors on symptoms and signs

A comparison is made between 35 patients with psychogenic traumata but no other factors observed to precede NCA here called the psychogenic group and 23 patients without psychogenic traumata where NCA followed infectious diseases or operative procedures, here called the convalescent group The frequency of some important symptoms and of normal findings in the work test and orthostatic test are given in table I As is to be expected psychic symptoms with anxiety were the dominating feature in 49 % of the psychogenic group but in 22 % only of the convalescent group The groups are small and the differences are not statistically significant.

Variations with regard to symptoms and signs appear regardless of the etiology though there was a tendency to preponderance of precordial pain and normal findings in the work test and orthostatic test in the psychogenic group

Hemodynamic studies by means of heart catheterization in patients with low physical working capacity

In NCA patients with low physical working capacity (PWC) are of special interest. The combination with ECG changes may arouse suspicion of myocarditis or ischemic heart disease. As a rule the case history gives valuable diagnostic guidance Moreover the ECG changes in organic heart disease are often localized in contrast to the diffusely spread sympathicotonic ECG changes in NCA. A combination of organic heart disease and NCA is, however rather common. In such cases and when the diagnosis is otherwise uncertain heart catheterization is necessary to elucidate the importance both of the functional and of the organic heart disease

Right-sided heart catheterization was performed in 17 patients with low PWC. The same characteristic pathophysiological syndrome was found in 15 patients, most of whom are described in earlier papers (17 18) They showed normal blood pressures in right atrium right ventricle, pulmonary artery and pulmonary artery wedge position Stroke volumes at rest as well as during work in relation to blood volume and body weight were normal, and there was no evidence of shunts. The cardiac output, however

was abnormally high in relation to the oxygen consumption, and a-v O difference was low in relation to pulse rate and cardiac output at rest as well as during exercise. The low PWC was thus explained by an insufficient oxygen utilization, and not by organic heart disease. The term vasoregulatory asthenia (VA)" has been proposed (17) because a disturbance of the adaptation of the peripheral circulation is considered the most likely cause of this pathophysiological syndrome. This syndrome corresponds to what has been previously called ergotrop (15) or hyperkinetic (8) disturbance of the circulation.

The same kind of disturbance has been observed previously with other methods. Among others Wolf and Wolff (46) made a protracted study of two healthy persons with Master' 2-steps test and ballistocardiogram. During emotional stress these persons showed increased minute volume at rest and after a standard work, and they complained on these occasions of palpitations, precordial pain, dizziness and fainting. Cohen et al. (5 7) found a lower endurance in NCA than in healthy people and a lower oxygen consumption at maximal work. This seems to correspond to the low PWC and low O consumption in relation to minute volume in our VA cases.

Heart catheterization in anxious patients was performed by Hickham et al. (16). Cardiac index was abnormally high at rest in relation to oxygen consumption. During work, however the relation became normal. Hickman also studied healthy students before and after examination. The majority of them got an increase of cardiac index together with elevation of pulse rate and blood pressure before examination, and afterwards they became normal. Only 3 students showed a decrease of cardiac index together with an increase of the peripheral resistance. These last cases seem to correspond to hypokinetic or trophotrop (15) circulation disturbance while the former group as well as VA" cases belong to the hyperkinetic type.

Stevenson et al. (38, 39) observed with Master's 2-steps test and ballistocardiogram that cardiac index was increased at rest as well as after work in patients with incidental or prolonged emotional disturbances, compared with relaxed patients. In one and the same person cardiac index decreased during relaxation, mainly because of decrease of pulse rate and at the same time PWC increased In mental depressions a disturbance of a hypokinetic type was observed with low cardiac index and stroke volume

In two of our catheterized patients the findings were aberrant and more difficult to interpret. Their circulatory condition during catheterization in the work test was not in accordance with precatheterization values they had tachycardia and lowering of the systemic blood pressure. The same disturbances, however were observed during their spontaneous attacks of heart troubles, and the findings may correspond to their condition during these attacks. The stroke volume was low but because of the tachycardia cardiac output was normal as was the oxygen consumption in relation to cardiac output. The pressures on the right side of the heart and in pulmonary artery wedge position were normal at rest as well as during maximal work, which seems to contradict a myocardial disease being the cause of the low stroke volume. Also in these two cases a disturbance of the peripheral circulation seems to be present, which possibly corresponds to a mingling of

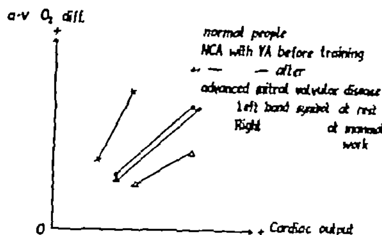


Fig. 1. Schematic diagram of the relationship between arteriovenous oxygen difference and cardiac output at rest and at maximal work in different conditions. It illustrates the cause of the low PWC in NCA cases with vasoregulatory asthenia and the effect of physical training. The physical working capacity is proportional to the a-v O difference multiplied by the cardiac output (that means the rectangle formed by the abscissa and the ordinate). For further explanation see text.

hyper and hypokinetic circulation disturbances. Fejfar (9) and Wolff (47) also pointed out that both these types of circulatory disturbances may occur in NCA.

Discussion

The finding during catheterization of hyperkinetic circulatory disturbances with low oxygen utilization in the majority of patients with low PWC might theoretically have two explanations 1 Inadequate distribution of the minute volume with superfluous circulation through shunts and areas with little demand for oxygen, and relatively little circulation through the capillaries of the working muscles. 2 Metabolic disturbances of the capacity for oxygen extraction in the muscle tissue.

Ad 1 The high correlation between marked orthostatic pulse reaction (OPR) and low PWC (28) supports a disturbance of the adaptation of the heart rate and the peripheral circulation which is also a plausible explanation of the more seldom observed hypokinetic type of circulatory disturbance. A marked orthostatic pooling of the blood cannot however in itself be the dominating cause of the low PWC in the routine bicycle test in the sitting position as the catheterized patients have been tested also in the lying position, and even then their PWC was low. Low PWC and marked OPR are thus, more probably reflecting different effects of the same disturbance. Symptoms such as cold hands and feet and numbness also fit well with disturbances of distribution of the blood flow. Thus the term "vasoregulatory asthenia" VA given to the hyperkinetic type of circulatory disturbances by Holmgren et al. (17) seems adequate.

Ad 2 In patients with paralytic myoglobinuria Larsson et al. (26) observed low a-v oxygen difference which did not increase during work. In Beri Beri with thiamine deficiency high cardiac output and low a-v oxygen differences are also observed. This may be due to both metabolic and hemodynamic disturbances (32). Faulty oxidative mechanism as well as high velocity of the blood flow and dilated arteriocapillary bed with arteriovenous shunts may contribute to the low oxygen utilization in this condition. To the best of the author's knowledge no other metabolic disturbances which might cause low oxygen utilization are known.

As shown by Holmgren et al. (19, 20) and Levander Lindgren (29) hard phys-

ical training normalizes the low PWC in VA. The increase of circulatory parameters such as total hemoglobin and heart volume during training is less important and can only partly explain the high increase of PWC. In one patient a further heart catheterization after training directly proved that a return of the oxygen utilization to normal was the cause of the high increase of PWC (19). As shown by Holmgren et al. (20) OPR decreases during physical training parallel to the increase of PWC. The most probable explanation of the effect of training seems to be a more adequate adaptation of the peripheral circulation.

A schematic review of the relation between a-v oxygen difference and cardiac output at rest and at maximal work, in healthy people and patients with VA before and after training is given in fig. 1. It is interesting to compare the relations in patients with advanced organic heart diseases, who often demonstrate abnormally high a-v oxygen difference as reported by among others, Holmgren et al. (21) in advanced mitral valvular disease. PWC is proportional to a-v oxygen difference multiplied by cardiac output at maximal work, that means maximal O_2 consumption which can easily be demonstrated in the figure.

The fact that a low PWC returns to normal after physical training suggests that lack of such training causes the low PWC. This is contradicted, however by following observations. 1. Statistical analysis has failed to show a significant correlation between immobalization and low PWC (28). 2. The degree of physical training which is necessary to bring the PWC back to normal in these cases is much higher than the usual degree of training in people with normal PWC. 3. Some people with low PWC evidently do not live a sedentary life. 4. The PWC can become normal even without physical training (29). With regard to the effect of physical training on vasomotor centres it is interesting to note the observation of Khristik (24) that the vasomotor reaction to cold returned to normal after physical training in hypertensive patients.

What might then be the etiology of the disturbance? Psychogenic influences are commonly regarded as being of importance in the development of VCA, and no significant qualitative difference with regard to symptoms and signs could be demonstrated in this paper between patients with and without obvious psychogenic traumata. It is a well known experience that in anxious people changes of the S-T segment and the T wave of sympathicotonic type occur (30, 34, 35, 36) and several observations also confirm that hyperkinetic circulatory disturbances may appear in these conditions (5, 7, 16, 38, 39, 46). It is therefore natural to consider corresponding findings in people with VCA to be pathogenetically analogous and caused by nervous influences on vegetative, vasomotor functions. Vasomotor hypothalamic centres are most probably engaged in the release of the disturbance (12, 31, 40) which is well in accordance with the subfebrile temperatures sometimes observed in these patients (13, 31, 40). In post-encephalitic and post-infectious cases there may be a structural or toxic damage of this centre or of substantia reticularis which modifies the impulses to it.

Summary

A brief review is given of etiological factors. Psychogenic trauma was the one most often observed (in 40 of 130 patients). With regard to abnormal findings in the

work test and orthostatic test there was no fundamental difference between a psychogenic and a convalescent group The pathogenesis and the etiology of the low PWC are discussed from findings at heart catheterization from statisti cally found correlations to marked orthostatic pulse reactions, and from the effect of physical training and clinical observa tions. It is concluded that low PWC, which is most often caused by a hyper kinetic type of circulatory disturbance, "vasoregulatory asthenia with a low arteriovenous oxygen difference, is most probably caused by disturbances of the adaptation of pulse rate and of the peripheral circulation Normalization occurs after hard physical training but also during other therapy and other correla tions indicate that immobilization can hardly be the essential cause of the low PWC. It is considered highly probable that low PWC is a sign of NCA per se like "sympathicotonic ECG changes and marked orthostatic reactions, and is mediated by nervous impulses on vasomotor hypothalamic centres.

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Treatment of Hyperlipidemia with d Thyroxine

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Hypercholesteremia continues to be considered one of the most significant factors promoting atherosclerosis, even if many questions concerning atherogenesis are still open. For this reason constant search is being made for a substance that would reduce the serum cholesterol level yet not have untoward side effects. Among hypocholesteremic agents recently used clinically one is d-thyroxine, the optical isomer of l-thyroxine, which is considered to have a low metabolic activity but to be clearly effective in reducing serum cholesterol.

In the present study the serum cholesterol and triglyceride levels were under observation in patients with essential hypercholesteremia during prolonged administration of d-thyroxine.

Material and methods

At the beginning of the test the series comprised 35 patients. During several months before the thyroxine administration, all the patients showed in most determinations a level of total cholesterol above normal. The starting values were in the range 289—685 mg %, most of them being over 350 mg %. The starting values for serum triglycerides showed greater variations than those for total-cholesterol. In one case the starting value was 49 mg %, in 3 cases 90—121 mg % and in all the other cases above 150 mg %, the maximum being 961 mg %. The age of the patients was in the range 36—68 years. There were 30 male and 5 female patients. None of the patients had had diabetes, nephrosis or disturbed thyroid function. However, a diagnosis of coronary occlusion had been made in 30 cases, arterial hypertension in 1 case, and coronary failure in 1 case. In 3 cases there were no objective symptoms.

The administration of d-thyroxine* was begun in all cases with a daily dose of 4 mg. 12 weeks later it was raised to 8 mg/day. The diet was unrestricted. At intervals of 3 weeks the serum cholesterol was determined by the method of Pearson et al. (13) and the glyceride glycerol was determined as triglyceride according to van Handel and Zilversmit (6).

Results

Dosage 4 mg

Clinical observations. All the patients tolerated the d-thyroxine well and none of them complained of nausea. Coincidentally with the d thyroxine administration one patient had a recurrent myo-

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* Dethyrona, Pharmacia (marketed as Choloxin® in Canada).

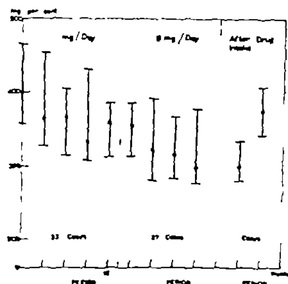


Fig 1 Change in serum total-cholesterol during and after administration of d-thyroxine. Determinations were made at intervals of 3 weeks. The administration of d-thyroxine was continued without interruption when the daily dose was raised from 4 mg to 8 mg The initial value on 8 mg/day was thus the same as the final value on 4 mg/day The median value of the group is shown by a circle and the end points of the line represent the values for the lower and upper quartiles.

cardial infarction which was fatal. In another case the stenocardiac distress disappeared completely two or three weeks after treatment with d thyroxine was begun and no nitroglycerin was necessary during the remainder of the observation period In the other cases the patients observed no difference in their subjective symptoms from the status before administration of the drug was started It was necessary to discontinue the drug in one case because of the onset of thyrotoxicosis of moderate severity 12 weeks after administration was begun. This patient had a nodular goiter and the stenocardiac pains were very severe during the hyperthyrosis. After discontinuation of d thyroxine and institution of imidazole treatment the hyperthyrotic symptoms disappeared within 2 weeks the stenocardiac pain disappeared almost completely and the laboratory results reverted almost to euthyrotic levels.

Response of cholesterol and triglyceride The mean value for serum total-cholesterol in 33 patients before administration of d-thyroxine was 400 mg % (fig 1) The dose of 4 mg/day during 12 weeks lowered the serum total-cholesterol in 27 cases. The change was statistically highly significant The dependence of the change in the cholesterol level on the starting value was examined by regression analysis (fig 2) which revealed a statistical significance ($P < 0.05$)

The dose of 4 mg/day did not produce a significant change in the serum triglycerides (fig 3)

DOSAGE 8 MG

Clinical observations Hyperthyrotic symptoms developed in two patients, in one of whom the stenocardiac pain was aggravated In neither case had the 4 mg dose clearly reduced the serum total-cholesterol. Before the onset of sweating tremor of the hands and tachycardia they had observed considerable loss of weight despite a good appetite. The other patients had no side effects nor did they observe any change in their subjective symptoms as compared with the pre treatment stage.

Response of cholesterol and triglyceride. When the dose was increased to 8 mg/day the serum total-cholesterol continued to decline. After the first three weeks the serum total-cholesterol was higher in 7 cases and lower in 21 cases than in the last determination during the 4 mg dosage. This already was statistically significant and the decline in serum total-cholesterol continued to predominate on continuation of the daily dose of 8 mg (fig 1) Comparison 9 weeks later of the dependence of the change upon the pre-treatment

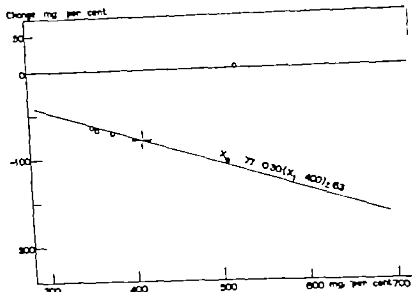


Fig. 2 Change in serum total-cholesterol after daily administration of 4 mg of d-thyroxine during 9 weeks. X_2 = change in mg %, X_1 = starting value in mg %. Dependence of change upon the starting value is statistically significant ($P < 0.05$)

Fig. 3. Change in serum triglycerides after daily administration of 4 mg of d-thyroxine during 9 weeks. The changes are insignificant.

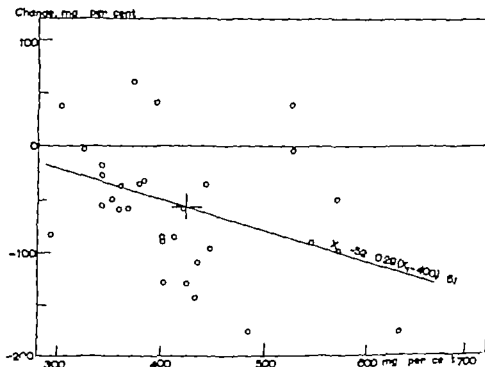


Fig. 4. Change in serum total-cholesterol after administration of 8 mg/day during 9 weeks, compared with cholesterol values before administration of d-thyroxine was begun. X_a = change in mg %. X_i = starting value in mg. Dependence of change upon the starting value is statistically significant ($P < 0.05$)

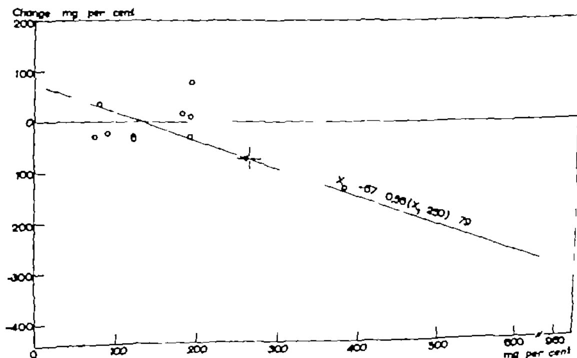


Fig. 5. Change in serum triglycerides after administration of 8 mg/day during 9 weeks, compared with values before administration of d-thyroxine was begun. X_a = change in mg %. X_i = starting value in mg %. Dependence of change on the starting value is statistically highly significant ($P < 0.001$)

values showed by regression analysis a statistically significant dependence ($P < 0.05$, fig 4)

The dose of 8 mg/day was found to produce a significant decline in the serum triglycerides. The change was dependent upon the starting values (fig. 5)

In the 3 cases in which the serum total-cholesterol level was not definitely influenced even by a dose of 8 mg, the dose was increased to 12 mg/day. However signs of hyperthyrosis became evident already during the first month in all of these cases. Concomitantly with the onset of thyreotoxicosis medicamentosa the serum cholesterol began clearly to decline.

Three weeks after discontinuation of d-thyroxine administration an upward trend was predominant in the serum total-cholesterol. However comparison of these values with those obtained in the last determination during the administration of 8 mg/day showed no statistical correlation.

Discussion

In seeking a hypocholesteremic agent suitable for clinical use the aim has been to find one that has as little side-effects as possible

The known effect of l-thyroxine in reducing the serum cholesterol cannot be put to use in euthyrotic hypercholesteremic subjects because of its marked metabolic activity. D-thyroxine has been proved to have clearly a hypocholesteremic effect but less calorigenic or cardiogenic effect.

The lowered serum cholesterol level during the use of d-thyroxine has been reported by many authors (1 2, 3 4 5 7 8, 9 10 12, 14). Our results are in agreement with those previously present ed in the literature. The decline in the serum total-cholesterol was significant with a daily dose of 4 mg of d-thyroxine and became more marked with the ad ministration of 8 mg/day. The dependence of the changes in the cholesterol level on the starting value was statistically significant when examined by regression analysis. However a question of decisive importance is whether or not the starting values reflect the real serum cholesterol level of the subjects. When the dose of d thyroxine is constant, probable differences between the starting cholesterol values of the different subjects are of more decisive importance than variations in the cholesterol values in one and the same subject. Relative changes, however which have been estimated by linear regression analysis only need not truly be the best description.

The changes caused by d thyroxine in the serum triglycerides did not follow the same trend as did those in the total cholesterol. A daily dose of 4 mg did not produce a clear change in the triglycerides whereas 8 mg/day had a significant lowering effect. So long as we do not know the role of triglycerides in the pathogenesis of atherosclerosis we are unable to state whether the effect of hypocholesteremic agents should be regarded as a cholesterol index or a triglyceride index". The reduction of the serum triglycerides by a higher dose of d-thyroxine than is needed for reduction of the cholesterol level may be a result of the concentration of d-thyroxine in certain organs, such as the liver and kidneys, and to a lesser degree in muscle and peripheral organs of the rat, which has been suggested as one of the reasons for also its lower calorigenic effect as compared to l thyroxine (15). On the other

hand the decline in serum triglycerides may be a sign of the onset of increased metabolic activity with increasing doses of d thyroxine (11) On the basis of the present results no definite conclusions can be drawn concerning the role of d thyroxine in the synthesis of lipids and the oxidation of fatty acids.

The most generally encountered side effect of d thyroxine when used as a hypocholesteremic agent may be considered to be its calorigenic activity Although it has not been possible to demonstrate either earlier or in the present study that hypermetabolism is common thyreotoxicosis medicamentosa may break out even at a low dosage, as occurred with a dose of 4 mg/day in one case in the present series. Naturally a hyper cholesteremic patient with cardiovascular disease must be held under close observation to avoid hypermetabolism during d thyroxine administration. A nodular goiter may be the trigger causing hyperthyrosis even with small doses of d thyroxine. Loss of weight appears to be the first sign of onset of hypermetabolism For this reason it may be well to weigh the patient at the time the serum lipid values are checked and to find the largest dose that does not cause loss of weight

The optimum dose of d thyroxine seems to be 4—8 mg/day In cases in which a still higher dosage is attempted when the above dose does not effect a reduction in the serum cholesterol, the desired effect will indeed be obtained but at the expense of still more marked hypermetabolism. Outside the present series, 2 patients with essential hyper lipemia were given increasing doses of d thyroxine. However the serum total cholesterol and triglycerides were unchanged The results of these two patients are not included in the present series, since their treatment was irregular

About 3 weeks after discontinuation of the d thyroxine administration an upward trend was predominant in the serum cholesterol level. It was not possible to prove by regression analysis the dependence of the increase on the values obtained at the last control examination during administration of 8 mg/day The interpretation of this would be that in cases in which d thyroxine reduces the cholesterol values to a relatively low level maintenance of this level is not as good as in cases in which the fall may numerically be greater but the level still remains high However the small size of the present series does not allow definite conclusions in this respect.

Summary

D thyroxine was administered to 33 patients with hyperlipidemia, and the serum total-cholesterol and triglyceride levels were observed at intervals of 3 weeks. A dose of 4 mg/day caused a significant reduction in the serum total-cholesterol after 9 weeks, and decline became more marked on a dose of 8 mg/day No change was observed in the serum triglycerides on a daily dose of 4 mg whereas 8 mg/day caused a statistically significant reduction.

Acknowledgements

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From the Departments of Toxoplasmosis and Viral Diseases and of Biophysics, Statens Seruminstitut, Copenhagen and Medical Department F (Head: M. Schwartz, M.D.) Copenhagen County Hospital, Glostrup, Denmark

Penicillamine Treatment in the Cold haemagglutinin Syndrome

By

K. Lind, B. Mansa and H. Olesen

The cold-haemagglutinin syndrome (C.A.S.) (4) is a rare, chronic disease in elderly persons which is characterized by a very high titre of cold haemagglutinins in the serum, by Raynaud's phenomena, and by a haemolytic anaemia. Raynaud's phenomena occur only when the patient is exposed to the cold and disappear again in the warmth. Exposure to the cold may provoke a haemolytic crisis, and may in some patients result in paroxysmal haemoglobinuria.

Cold agglutinins, acting on the patient's red cells, are constantly present in titres from several thousands up to one million. They have a high thermal amplitude, i. e. they still agglutinate the red cells when the temperature is raised to 25–30° C, though with a lower titre than at + 4° C. This phenomenon is the main factor in the pathogenesis of the Raynaud symptoms (16) and it can be demonstrated in vivo by microscopy of the patient's conjunctival capillaries under cooling (11).

Both the degree of the haemolytic anaemia and the intensity and frequency of the haemolytic crises may vary considerably from patient to patient. This variation depends mainly on the thermal amplitude of the cold agglutinins and on the extent of the exposure to the cold. Dacie, Crookston and Christenson (6) have shown that the direct Coombs' test is positive when an anti-non-gamma globulin is used for the reaction and that the cold agglutinin acts as a haemolysin when complement is present. This was later confirmed by Lewis, Dacie and Szur (14) who investigated the mechanism of haemolysis in vivo after the patient's ^{51}Cr-labelled erythrocytes had been exposed to her own high-titre cold agglutinin in vitro under different conditions allowing complement adsorption. *Jonsen and Kåss (12) showed that in* the C.A.S. complement is consumed during the process of haemolysis in vivo.

No specific treatment of the C.A.S. is available (5). Steroids, cytotoxic agents (e. g. urethane, nitrogen mustard) and radiation therapy (thorotrast, radioactive gold, X-ray) have been tried with the aim *of reducing* the production of cold

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Table I. Method of titration for cold agglutinins

	PRIMARY dilution				SECONDARY dilution				TERTIARY dilution				
I	0.90 serum 1.98 saline 2.88 (=1 3.2)			→	0.2 PRIM. dil. 3.0 saline 3.2 (=1 51.2)			→	0.2 SEC. dil. 3.0 saline 3.2 (=1 819.2)				etc. →
II	↓	0.6	0.3	0.2	↓	0.6	0.3	0.2	↓	0.6	0.3	0.2	
Saline		0.6	0.9	1.4		0.6	0.9	1.4		0.6	0.9	1.4	etc. →
Serum dilution	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	
III Red cells.	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	etc. →
Final titre	4	8	16	32	64	128	256	512	1,000	2,000	4,000	8,000	

For titration in albumin saline is replaced by 30 % bovine serum albumin, diluted 1:5 in saline. Red cells = 0.8 % suspension in saline.

Three parallel serial dilutions similar to III can be set up from II.

the tube just before as + the titre of the ± reading as added in brackets. To control the reversibility of the reaction, reading as performed after incubation at 37° C for 30 minutes.

Significance. In previous tests using this method 4-fold difference in titre was shown to be significant.

COMPLEMENT TITRATION

Complement titration was carried out according to the method of Pillemer et al. (19) with the modification that one unit of complement was fixed as the minimum amount of serum necessary to produce 50 % haemolysis of the sensitized sheep cells. All sera were titrated in one experiment, the experiment being repeated next day.

TEST FOR HAEMOLYSINS

The sera of case 1 were tested for haemolysins (I) in the patient' fresh serum, (II) in the patient inactivated serum (56° C/30 min.) alone, and (III) with guinea-pig serum added. The sera were acidified by adding 1/10 olume of 0.2 N—HCl (4). They were tested with ( ) the patient cells washed at 37° C and (b) normal group-0 cells. One volume of a 50 % suspension of the cells was added to 10 volumes of the sera. The tubes were incubated 90 min. at 37° C and read after centrifugation. The sera of case 2 were tested in the same way except that fresh normal serum was used instead of guinea-pig serum, and there was a supplementary test with normal group-0 trypsinized cells (18). Furthermore, the tubes were incubated for two hours instead of 90 minutes.

ANTIGLOBULIN SERA

Sera from rabbits repeatedly immunized by intraperitoneal and intravenous injections of fresh human group-0 serum were used as antiglobulin sera. The sera were inactivated at 56° C for 30 min. and were then repeatedly absorbed with washed human erythrocytes of group-A_1 and -B until the undiluted sera no longer agglutinated the normal (unsensitized) cells. When appropriately diluted they strongly agglutinated cells sensitized by cold agglutinins or incomplete Rh-antibodies. The antiglobulin reaction was evaluated by serial two-fold dilutions of the antiglobulin serum in saline and in an 0.1 % solution of human gamma-globulin in saline added to neutralize the anti-gamma-globulin.

agglutinins but without success. Sympathectomy vasodilator drugs, and anticoagulants are of no or of only transient value. If a blood transfusion is indicated washed red cells should be preferred to avoid donor's complement which may accelerate the process of haemolysis.

As there is no specific treatment available for reducing the production of cold agglutinins a symptomatic treatment aiming either at a destruction of the cold agglutinins or at a reduction of their activity as agglutinins or haemolysins might be attempted

Cold agglutinins are macroglobulins (9) i. e. proteins with a molecular weight of approximately one million. Deutsch and Morton (7) have shown that macroglobulins are dissociated to components of lower molecular weight by mercaptans. If cold agglutinins are subjected to this treatment *in vitro* the dissociation is followed by a concomitant reduction of the cold agglutinin activity (8) Recently Ritzmann and Levin (20) treated a patient with the C.A.S with mercaptans and found a reduction in the cold agglutinin titre and a prolongation of the survival time of ^{51}Cr labelled erythrocytes during treatment with D L-penicillamine.

It was therefore decided to treat two patients with typical C.A.S with D-penicillamine a relatively stable mercaptan with a very low toxicity (22) after its effect on the patients sera *in vitro* had been proved.

Material and methods

SERA

Blood samples were taken in warmed (37° C) tubes, and after clotting and centrifugation, both at 37° C, the sera were used the same day or stored at — 20° C. Sera used for titration of complement were sto — 70° C in sealed ampoules until just use.

RED CELLS

Red cells were obtained from blood co in tubes containing 1 mg heparin per n the Coombs and haemolysin tests th were washed in saline at 37 C.

COLD AGGLUTININ TITRATION

The red cells were obtained alternately three donors (group-0 Rh pos.) These had been bled for the routine cold aggl tion test at Statens Seruminstitut throu more than 5 years and their red cells l constant and equal agglutinability The was collected in equal volumes of gl citrate (4.5 glucose plus 3.8 % so citrate, filtered and autoclaved) and at 4 C from 24 hours to 7 days in this tion. Immediately before use the cells washed twice and an 0.8 % suspensio packed cells in saline was made.

Titration. Twofold dilutions of the were made using the method indicate table I Most of the sera were diluted in saline and in a 6 solution of bovine se albumin in saline this albumin milieu shown an optimum enhancement of the tion and a more distinct endpoint res (15) The cell suspension was then ad the final concentration of cells in each thus being 0.16 % in a volume of 0.4 Tubes measuring 70 by 5 mm were used. tubes were shaken and then incubated o night at 4 C. Three parallel dilutions prepared from each serum and were e g for the comparison of incubation at ferent temperatures. All titrations were m by the same technician.

Reading Immediately after being remo from the refrigerator the tubes were read w the naked eye by the light of a screened 60 frosted bulb. After gentle shaking the stren of the agglutination was recorded as ++ if the cells formed an unbroken button, ++ if big pieces and coarse grains were served as + if small grains were seen, as ± if the grains were just visible. The ti was expressed as the reciprocal of the highe dilution in which agglutination was st visible. If the end point was read as ± ar

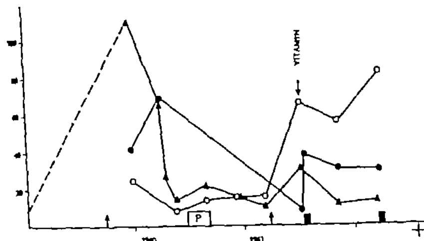


Fig. 1 Clinical and laboratory data in case 1 ↑ = Exposure to cold. ▮ Hepatic coma. ▲ = Glutamic-pyruvic-transaminases. ● = P P time. ◯ = Icterus index. P Penicillamine

of the ileum. As no thrombosis was found in the resected part of the ileum, the pathological process was presumed to ha been more centrally localized. From the time of the operation until the patient died on Feb. 18, 1961 the faeces were thin with increased lipid content, despite treatment with Zymopan ®

From 1958 until Nov 1960, the Raynaud' phenomena appeared when the patient was exposed to temperature below 18° C. From then on her sensitivity to cold weather increased steadily and at times her bedroom had to be specially heated. During a short stay at home the patient was exposed to the cold, and she was readmitted on Nov 28, 1960, with high temperature, icterus, and laboratory data indicating a severe affection of the liver (lowered P P-time, and extremely elevated glutamic-pyruvic transaminases) Her condition improved slowly and the icterus diminished, but after a renewed exposure to the cold during short stay at home, her condition again deteriorated and the patient was admitted to hospital on Jan. 14 in hepatic coma with typical clinical symptoms and changes in the electroencephalogram She was treated with glucose and terramycin intravenously and the hepatic coma subsided. After another attack of hepatic coma on Feb. 6, 1961 which this time began spontaneously without preceding exacerbation in the haemolytic state, the patient improved only slightly with the same treatment. The icteric state remained unchanged, the patient felt extremely tired and was unconscious at times. On Feb. 16, tw days before her death, oedema and deep cyanosis developed in the face and in the extremities, and no blood could be drawn from th peripheral vessels (fig 1)

Somatic status Before the patient became critically ll, e. in Nov 1960, she looked aged, tired and pale w th an icteric tinge. Liver spleen and lymph nodes were not palpable and no gangrene was found in the fingers or toes.

Routine laboratory investigations Haemoglobin 7 5—11.8 g %, Haematocrit 23—44 %, Red cells 2 720 000 Differential count Normal. ESR at 37 C 40 mm/hour Reticulocytes: 1—48 per thousand Sternal puncture Normoblastic, hyperplastic erythropoiesis. Haptoglobin 0.

At the *autopsy* the liver was found to be greatly diminished in size (21 × 14 × 3.5 cm) the shape was normal and the surface smooth without lobulation. Microscopy revealed extreme changes with necrosis of most of the liver-cells and greatly increased interstitial fibrosis. *Diagnosis* Extreme necrosis and incipient cirrhosis of the liver Bone marrow Moderate hyperplastic haematopoiesis. Nothing remarkable in the other organs (E. Bredahl).

ANTIGLOBULIN TESTS

The sensitization of the red cells is described in the section dealing with the *in vitro* experiments. The test was carried out in accordance with Coombs et al. (3)

EUGLOBULIN PRECIPITATION

After thorough washing in distilled water centrifuge tubes were dried for 24 hours at 180° C and weighed twice on a Mettler balance with an accuracy of 0 01 mg Two ml serum was diluted in 25 ml distilled water in the centrifuge tubes and centrifuged for 30 min. at 2 000 × G The supernatant was discarded and the tubes dried at 180° C for 24 hours. The difference between the weight before and after euglobulin precipitation was corrected by means of a blind control and expressed as mg euglobulin precipitated per ml serum Plastic gloves were worn by the operator Unheated precipitates were redissolved in a volume equal to the original amount of serum for further tests.

ERYTHROCYTE SURVIVAL

Erythrocytes from the patient were labelled with ^{51}Cr at 20° C by the method of Mollison and Veall (17) Twenty ml blood was collected in sterile acid-citrate-dextrose anticoagulant and the washed erythrocytes labelled with 45 μc of $Na_2{}^{51}CrO_4$ After washing at room temperature with saline the suspended cells were injected into the patient and blood samples were taken after 15 min., 2 hours and subsequently at intervals of one or two days for one month All blood samples collected were measured in a well-scintillation counter on the same day The activities were expressed as a percentage of the activity measured in the sample taken after 15 min. ^{51}Cr $T^1/_2$ was not corrected for ^{51}Cr-elution from the erythrocytes. The normal value was 28 ± 2 days.

PAPER ELECTROPHORESIS

was performed in a Grassman type apparatus (horizontal strips) with Schleicher and Schull 2043 B paper strips. The TRIS buffer of Aronsson and Gronwall (1) was used. The dried strips (110° C, 30 min.) were stained with Amidoblack 10 B The Spinco Analytrol was used for quantitative evaluation.

IMMUNO-ELECTROPHORESIS

The micromethod described by Scheidegger (21) was used throughout with only slight modifications. Buffer Sodium barbiturate with calcium lactate, pH 8.6 (10) The following antisera were used for the development of the precipitates 2 pools of rabbit anti-human serum, a rabbit antiserum which mainly contained antibodies to human gamma globulin, and horse anti-human serum (No. 13414) from Institut Pasteur Paris. The washed and dried plates were stained with Amidoblack 10 B.

ANALYTICAL ULTRACENTRIFUGATION

The ultracentrifugal analysis was conducted in the Spinco Model E centrifuge with the analytical rotor A at 59780 rpm. The sera were diluted with phosphate-buffered saline, pH 7 38 to contain about 1 % protein. To determine the proportions of the components the Schlieren patterns were enlarged five times and traced on to graph paper planimetry was then performed.

PENICILLAMINE

D-penicillamine = (+)-dimethylcysteine HCl, (The Distillers Company Ltd., London) was dissolved in saline and neutralized by adding NaOH for use *in vitro*. Penicillamine was given to the patients in capsules.

DIALYSIS

Dialysis was carried out against saline or buffered saline at 4 C using Visking tubes No 24/32

BUFFERED SALINE

Saline = 0.15 M NaCl solution in redistilled water Buffered saline = Sorensen phosphate buffer made isotonic with NaCl.

Case reports

Case 1 A 66-year-old unmarried housekeeper who was operated on for an ovarian cyst in 1949 Since 1956 the patient had suffered from typical attacks of Raynaud's phenomena followed by haemoglobinuria. The diagnosis of cold-haemagglutinin syndrome was established in hospital in 1958. In July 1960 acute laparotomy was performed and a 100 cm section of the small intestine was resected owing to widespread gangrene

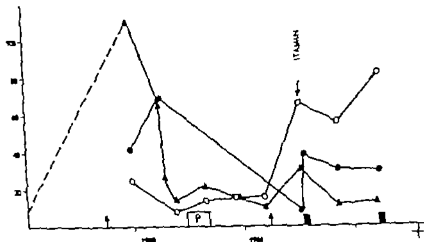


Fig. 1 Clinical and laboratory data in case 1. ↑ = Exposure to cold. ▮ Hepatic coma. ▲ = Glutamic-pyruvic-transaminases. ● = P-P time. ○ = Icterus index. P Penicillamine.

of the ileum. As no thrombosis was found in the resected part of he ileum, the pathological process was presumed to have been more centrally localized. From the time of the operation until the patient died on Feb. 18, 1961 the faeces were thin with increased lipid content, despite treatment with Zymopan®.

From 1958 until Nov 1960, the Raynaud' phenomena appeared when the patient was exposed to temperature below 18° C. From then on her sensitivity to cold weather increased steadily and at times her bedroom had to be specially heated. During a short stay at home the patient was exposed to the cold, and she was readmitted on Nov 28, 1960 with high temperature, icterus, and laboratory data indicating severe affection of the liver (lowered P-P-time, and extremely elev ted glutamic-pyruvic transaminases). Her condition improved slowly and the icterus diminished, but after renewed exposure to the cold during short stay at home, her condition again deteriorated and the patient was admitted to hospital on Jan. 14 in hepatic coma with typical clinical symptoms and changes in the electroencephalogram. She was treated with glucose and terramycin intravenously and the hepatic coma subsided. After another attack of hepatic coma on Feb. 6, 1961 which this time began spontaneously without preceding exacerbation in the haemolytic state the patient improved only slightly with the same treatment. The icteric state remained unchanged, the patient felt extremely tired and was unconscious t times. On Feb. 16, two day before her death, oedema and deep cyanosis developed in the face and in the extremities, and no blood could be draw from the peripheral vessels (fig 1).

Somatic status. Before th patient became critically ill, in Nov 1960, she looked aged, tired and pale with an icteric tinge. Liver spleen and lymph nodes were not palpable and no gangrene was found in the fingers or oes.

Routine laboratory investigations. Haemoglobin 7.5—11.8 g %, Haematocrit 23—44 %, Red cells 2,720,000 Differential count Normal. ESR t 37° C 40 mm/hour Reticulocytes 1—48 per thousand. Sternal puncture Normoblastic, hyperplastic erythropoiesis. Haptoglobin 0.

At the *autopsy* the liver was found to be greatly diminished in size (21 × 14 × 3.5 cm) the shape was normal and the surface smooth without lobulation. Microscopy revealed extreme changes with ecrosis of most of the liver-cells and greatly increased interstitial fibrosis. *Diagnosis* Extreme necrosis and incipient cirrhosis of the liver. Bone marrow Moderate hyperplastic haematopoiesis. Nothing remarkable in the other organs (E. Bredahl).

Table II Influence of different concentrations of penicillamine on cold agglutinin titre

Concentration of penicillamine in mM	Cold agglutinin titre
125.0	256
12.5	4 000 (8 000)
2.5	8,000 (16,000)
0.5	32,000 (64 000)
0.1	32,000 (64,000)
Control	64 000

Plasma (case 1) was incubated with neutralized D-penicillamine at 37 C for 5 / days. The t tra tion was performed in normal serum diluted 1 5 in saline.

Table III Case 2 Changes in cold agglutinin activity during penicillamine treatment

Cold agglutinin serum	Duration of treatment	
	3 / hours	48 hours
Treated	*100 000*	*1,600*
Untreated	300 000	150,000

Equal parts of serum diluted 1:25 in buffered saline and neutralized penicillamine (80 mM) were incubated at 37 C.

Case 2. An 85-year-old retired head nurse who was operated on for a fibroma uteri in 1920. For the last 20 years she had suffered from anaemia with haemoglobin values vary ing from 55 to 80 but without any subjec tive symptoms. In 1955 for the first time, typical Raynaud s phenomena were observed on exposure to the cold, and on one occasion she passed black" urine. A very high titre of cold agglutinins was found in her serum and the diagnosis C.A S was established. The following data were obtained from the medical department where the patient was admitted seven times during the period from Jan 1956 to Aug 1961

From time to time the patient had been treated for anaemia with iron, liver and cyanocobalamin-preparations from Sept. 20 until Dec. 25 1960, meticorten was given, without any appreciable influence on the symptoms from the C.A.S. Since Oct. 25 1960 Durabolin® (25 mg a week) had been administered

Mentally and somatically the patient was well preserved she was pale and subicteric on returning from outdoors pronounced acro-cyanosis could be observed. Liver spleen and lymph nodes were not palpable.

Routine laboratory investigations. Blood pic ture Red cells 1 680 000—2,950,000/mm³ haemoglobin 7 0—10.6 g/100 ml haema-tocrit 22—28 % reticulocytes 0.5—6.4 % leukocytes 6 400—15,000 differential count Normal. ESR 94 mm/hour at 37° C (Wester green) Sternal puncture Normoblastic hyper plastic erythropoiesis, lymphocytes 2 % plas-ma-cells 1 % Serum bilirubin 0.9—4 7 mg/ 100 ml (< 1 0) Serum haptoglobin 0—0. Glutamic pyruvic transaminases 0.4—0.3 units per ml (< 1.5) Urine Benzidine test negative on all occasions.

Results

IN VITRO EXPERIMENTS

Influence of different concentrations of penicillamine on the cold agglutinin titre (table II)

Dilutions of neutralized penicillamine in saline were incubated with equal volumes of plasma under liquid paraffin at 37 C for 5 1/2 days. The results in-dicate that 2.5—12.5 mM penicillamine gives a significant reduction in the cold agglutinin titre.

Influence of time on the penicillamine effect on cold agglutinin activity and on the possible restoration of this activity in the absence of penicillamine

Serum (case 2) diluted 1 25 in buf fered saline, pH 7.38, was incubated with an equal volume of neutralized penicillamine the final concentration being 40 mM. Control serum was in-cubated with buffered saline alone. After 3 1/2 hours and again after 48 hours specimens were removed, dialysed over

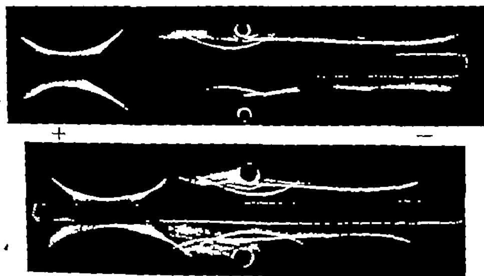


Fig. 2. Immuno-electrophoretic investigation of sera from case 1 a) before, and b) after the in vitro treatment with penicillamine, and from case 2, c) before, and d) after the in vitro treatment. The precipitates were developed by means of the pooled rabbit anti-human serum. Note the change of the β_{2M}-line caused by the treatment; the position closer to the antibody trough is caused by the higher diffusion rate of the dissociated protein.

night and tested for cold agglutinin activity. The serum was incubated and dialysed at 37° C.

Table III shows that the reduction in the titre was greater after an incubation for 48 hours than after an incubation for 3 1/2 hours.

After being dialysed at 37° C the two 48-hour specimens were left in order to ascertain whether a possible restoration of the cold agglutinin activity had taken place in the absence of penicillamine. After 6 days the titres were unchanged for both treated and untreated specimens (1,600 and 200,000 respectively).

Effect of penicillamine on cold agglutinin sera shown by immuno-electrophoresis

Immuno-electrophoretic analysis of serum from patient No. 1 with anti-human serum from rabbit and horse shows the development of the β_{2M}-line as a rather dense line (fig. 2 a) indicating an increased concentration of the component. The mobility of the main part of this globulin is less than for normal β_{2M}-globulin. The broad line continues as a fainter line which reaches the point of application and even beyond that into the α_2-region. The identity (1 A) is indicated by the change in position and shape observed when in vitro penicillamine treated serum is analysed (fig. 2 b). The line is then seen near the antibody trough as a strongly curved line resulting from the increased diffusion rate of the dissociated β_{2M}-globulin.

Immuno-electrophoretic investigations of sera from patient No. 2 show a dense β_{2M}-precipitate (fig. 2 c). The electrophoretic position of the line is identical with that of the β_{2M}-line in normal sera. Sera treated with penicillamine showed changes in the position and shape of the β_{2M}-line similar to those described for the sera from patient No. 1 (fig. 2 d).

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Influence of time on the penicillamine effect on cold agglutinin activity and on the possible restoration of this activity in the absence of penicillamine

Serum (case 2) diluted 1 25 in buffered saline pH 7 58, was incubated with an equal volume *of neutralized* penicillamine the final concentration being 40 mM Control serum was incubated with *buffered saline* alone. After 3 1/2 hours and again after 48 hours specimens were removed dialyzed over

Table V Analysis of sera from two patients with the cold-haemagglutinin syndrome before during and after treatment with penicillamine

Patient no. 1 was treated in the period 16–22/12 1960 and patient no. 2 in the period 26/1–3/2 1961

Pat. no.	Date	Euglobulin (mg/ml serum)	Paper electrophoresis % of total protein					Ultracentrifugation % of total protein			Cold agglutinin titre in albumin medium
			Alb.	α	α	$\beta+\beta$	$\gamma+\gamma$	4 S	7 S	19 S	
1	11/11 1960		61.6	4.8	6.4	11.4	15.8	86.9	7.1	6.0	1 000 000
	15/12 1960	2.98	50.7	6.4	8.5	9.6	24.8	73.0	16.5	10.4	384,000
	19/12 1960	3.93	49.3	7.1	6.7	9.3	27.6	69.1	19.2	11.7	1,500,000
	16/2 1961	5.98	41.2	6.3	7.1	8.0	37.4	61.9	22.8	15.3	500,000
2	29/9 1960	–	66.6	4.8	5.5	8.1	15.0	81.6	11.9	6.5	98,000
	16/1 1961	–	64.6	5.9	5.7	5.9	17.9	83.9	6.8	9.3	384,000
	2/2 1961	–	64.1	6.1	6.3	7.1	16.4	81.1	9.0	9.9	512,000

6/12 1960 2.59 mg euglobulin ml serum.

Case 2 For 11 days total of 31.5 g D-penicillamine was given in daily doses of 5 × 4 capsules on the last day however only 5 × 2 capsules were given.

Both patients were kept t a constant room temperature during and after the treatment.

RECORD OF TESTS PERFORMED BEFORE, DURING AND AFTER THE THERAPEUTIC TRIALS

Cold agglutinin titre

Taking the spontaneous variations before this period into account, the titre in both patients remained unchanged during and after the period of treatment. Nor were there any changes in the thermal amplitude of the cold agglutinins in any of the patients (fig 3 and 4)

Ultracentrifuge patterns (table V)

Case 1 Increasing concentrations of the 19 S component and of the 7 S component were found during the examination period and no decrease occurred during the treatment with penicillamine.

Case 2 No change in the 19 S component was found during the treatment.

Paper electrophoresis (table V)

Case 1 The distribution of proteins, determined by paper electrophoresis, in the sera from this patient underwent a marked change during the whole period of investigation From a nearly normal distribution an increasingly severe hyper gamma-globulinaemia developed Simultaneously a corresponding decrease in albumin content was demonstrated. The amount of α_1-globulins present showed a consequent increase.

Case 2 The distribution of components n sera from this patient fell within the normal ranges, except for the elevated α-globulins. A narrow peak with γ_1-mobility could be seen. The concentration of this component is estimated as one third of the total γ-globulins. No change was found in either of the patients during the treatment with penicillamine.

Immuno-electrophoresis

During the period of treatment the β_{2M}-precipitation line of the sera from

Table II Antiglobulin test. Effect of penicillamine on the sensitization of red cells with cold agglutinin serum and fresh normal serum

Fresh normal serum 0.2 ml	Cold agglutinin serum 0.2 ml			
	Untreated not dialysed, titre 16 000	Untreated dialysed, titre 24 000	Treated not dialysed, titre 6 000	Treated dialysed, titre 4 000
Untreated not dialysed	++	Not tested	0	++
Untreated dialysed	++	+(+)	Not tested	+
Treated not dialysed	0	Not tested	Not tested	0
Treated dialysed	+(+)	+	Not tested	+
Untreated not dialysed Untreated dialysed Treated dialysed	(0.4 ml, control)	0		

0.4 ml of the combined sera were incubated with normal red cells at 22 C for 2 hours. The cells were washed at 37 C and tested with an antiglobulin serum.

Ultracentrifugation analysis of the effect of penicillamine on cold agglutinin sera

Sera from both patients were analysed in the ultracentrifuge at the same dilution before and after the *in vitro* treatment with penicillamine. The 19 S proteins were clearly seen in the untreated sera whereas they were barely visible after treatment at the same time the relative concentration of 7 S material increased.

Effect of penicillamine on the sensitization of red cells with cold agglutinins

Serum from patient No 2 was treated with small amounts of penicillamine reducing the cold agglutinin titre to about 25 per cent. Fresh normal serum was treated in the same way Buffered saline instead of penicillamine was added to the untreated sera Half of each serum both the treated and the untreated was dialysed against buffered saline pH 6 4 Normal group-0 red cells were incubated with combinations of the different samples at 22 C for 2 hours at this temperature fresh normal serum alone was unable to sensitize normal red cells. After washing in saline 4 times at 37 C, the cells were tested with the antiglobulin serum The results are shown in table IV

This experiment shows that sensitization cannot take place in the presence of penicillamine even though sufficient cold agglutinin and fresh serum are present. The weaker reactions of cells sensitized with a mixture of the two sera, both of which were dialysed might be due to a depletion of Ca^{++} and Mg^{++}

If the patient s red cells (No 2) washed 4 times in 37 C saline, were incubated with penicillamine as described above, their ability to react with the antiglobulin serum was unchanged i e. once sensitized the red cells are not desensitized by penicillamine

THERAPEUTIC TRIALS

Case 1 For 7 days a total of 9 6 g D-penicillamine was given in daily doses of 5 + 2 + + 2 + 3 capsules, each of 150 mg on the last day only 4 capsules were given. Probenecid was administered for 7 days, starting two days before, the total dose being 6.25 g the intention was to reduce the urinary excretion of penicillamine.

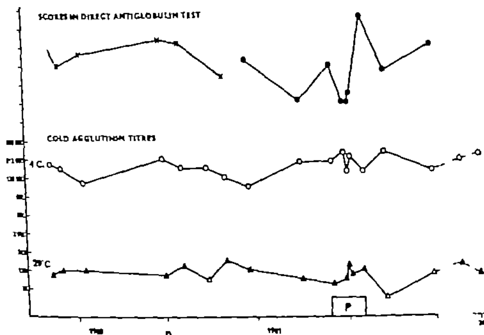


Fig 4 Case 2. Cold agglutinin titres and scores in direct antiglobulin tests in patient with cold agglutinin syndrome. P = penicillamine treatment with 31.5 g in total.

day to day No clear-cut changes in the haemolysin tests were recorded during penicillamine treatment.

Case 2. The serum contained haemolysins which reacted with normal group-0 cells and normal trypsinized cells in the presence of complement. The patient's red cells were haemolysed by fresh normal serum. These tests showed no greater variations during the treatment with penicillamine.

The *Donath-Landsteiner* test was constantly negative for both patients during the periods of investigation.

Complement titrations were carried out on sera from *case 2* only (fig 5) These sera all showed significantly low titres, containing less than sixteen 50 % haemolytic units per ml. Three sera from a normal person (EL) had been kept sealed at −70° C for the same period as the first serum from case 2 they contained 100 83 and 77 units, respectively Another normal serum (MAN) tested when fresh, contained 100 units. It is remarkable that all sera from the period of penicillamine treatment contained less than 5 units. Only one specimen after that period namely from July 26 showed the same low value.

Erythrocyte survival

$T^{1}/_{2}$ for Cr-labelled erythrocytes in case 1 was found somewhat shortened, i. e. 16.5 days (fig 6) No change occurred in the curve during treatment with penicillamine.

Discussion

The sera of the two patients contained high-titre cold agglutinins which by ultracentrifugation were demonstrated in the

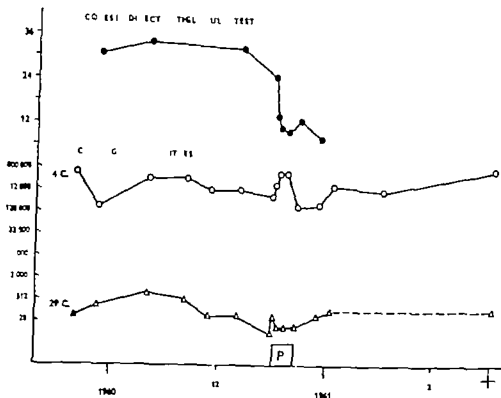


Fig 3 Case 1 Cold agglutinin titres and scores in direct antiglobulin tests in a patient with cold agglutinin syndrome P = penicillamine treatment with 9 6 g in total

both patients did not show any changes in shape or position similar to those described under *in vitro* experiments.

Euglobulin precipitation

Case 1 During the examination period increasing amounts of euglobulin were precipitated corresponding to the changes found by ultracentrifugation and paper electrophoresis. This euglobulin contained the major part of the cold agglutinin activity (table V)

Case 2 In this patient the euglobulin test was negative.

Sensitization of red cells

The sensitization of red cells from heparinized blood samples was evaluated according to their reaction in dilutions of an antiglobulin serum The reactions were scored in accordance with Callender and Race (2)

Case 1 The red cells of this patient showed a much weaker reaction in the antiglobulin test than those of case 2 A drop in scores coincides with the period of treatment (fig 3)

Case 2 No change in scores was observed. The two antiglobulin sera used gave identical scores (fig 4)

Haemolysins

Case 1 The serum contained haemolysins active against normal red cells, the reaction being stronger with the addition of complement. The patient s serum haemolysed her own red cells this reaction was much weaker and varied from

effect was observed either in case 1 who received 1.5 g a day for 7 days, or in case 2, who received 3 g a day for 11 days. The patient treated by Ritzman and Levin was given 1.5 g a day for 10 days an effect being obtained on the cold agglutinin titre and the erythrocyte survival.

The penicillamine used by Ritzman and Levin was D,L-penicillamine, while the preparation used in this study was D-penicillamine. The qualitative effect *in vitro* of the two drugs seems to be similar. *In vivo* experiments with L-penicillamine in animals have shown that this preparation has an anti-pyridoxine effect (13). The discrepancy in the clinical effect obtained in the two studies might be caused by the antimetabolic effect of D,L-penicillamine used by Ritzman and Levin. This explanation, however is less probable, as the fall in the cold agglutinin titre obtained in the patients treated by these authors occurred immediately after the administration of the drug.

The clinical and laboratory data for both patients are typical for the cold agglutinin syndrome. However in case 1 the final development of signs of increasing liver cell destruction seems unusual. Simultaneously a significant increase in γ-globulins, macroglobulins, and euglobulin was recorded without a corresponding increase in cold agglutinin activity (table V). Fig. 1 shows that the first and second attacks of liver insufficiency occurred a few days after a severe exposure to cold weather followed by haemolytic crises. The third and fatal attack was not preceded by exposure to the cold but at that time the patient presented marked Raynaud's phenomena even in a well-heated room. While the first exposure to the cold was followed by an enormous increase in glutamic pyruvic transaminases and a moderate increase in the Meulengracht icterus index, the reverse was the case after the second exposure. A relationship between the increased liver cell destruction and the periods with an increased rate of haemolysis seems probable. The pathogenesis of this development of liver cell destruction is not clear.

It should be noted that the penicillamine treatment did not seem to interfere with the course described.

The only change recorded in connection with the penicillamine treatment was the low complement titres found in case 2 (fig. 5). This effect might be ascribed to the treatment, if the influence on complement seen in the *in vitro* experiments is taken into account. The drop in scores from the direct antiglobulin tests of case 1 (fig. 3) is difficult to evaluate when compared to the scores in case 2 (fig. 4).

The possible relation between the sensitization of red cells demonstrated in the antiglobulin test and the titres of complement and cold agglutinins was examined in other patients with the cold agglutinin syndrome. The material is not yet sufficiently investigated to draw any conclusions.

Summary

The clinical and laboratory findings are described in two female patients with the cold agglutinin syndrome. There was no recorded effect of the treatment with D-penicillamine on the clinical state or on the laboratory findings, i. e. cold agglutinin titre ultracentrifugal and immuno-electrophoretic results. In the *in vitro* experiments, on the other hand the effect of D-penicillamine was clearly shown by a reduction in the cold agglutinin titre and in the 19 S proteins, by changes in the position and shape of the β_{2M}-line, and by a "blocking" effect

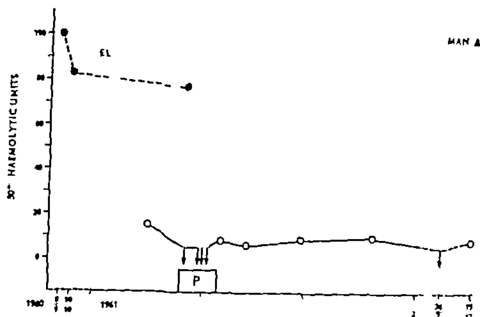


Fig 5 Complement titrations of sera from case 2 Arrows indicate titres lower than 5 units. P = penicillamine treatment. EL = normal serum kept frozen. MAN = fresh normal serum.

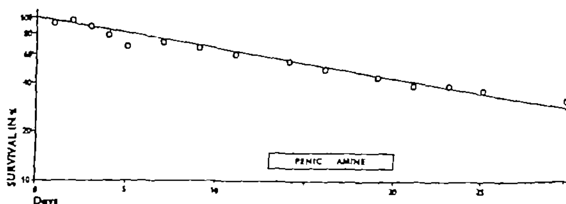


Fig 6 Erythrocyte survival in case 1 ^{51}Cr T½ = 16.5 days.

19 S globulin fraction. The qualitative effect of the D-penicillamine on the cold agglutinins was clearly shown *in vitro* by a decrease in the cold agglutinin titre together with a reduction in the concentration of 19 S globulins. A corresponding effect was shown in immuno-electrophoresis of the sera by the change in position and shape of the β_{2M}-line. Furthermore a "blocking effect of penicillamine on the sensitization of normal red cells by cold agglutinins and complement was demonstrated *in vitro* From these data it was assumed that a clinical effect both on the agglutinating activity and on the sensitizing activity of the cold agglutinins might be obtainable

It was difficult however from the *in vitro* experiments to assess the clinically effective dose of penicillamine, as the fate of this compound in the organism was not fully elucidated No attempt was made to determine the concentration of penicillamine in sera or excretions during the administration.

The results of the *in vitro* experiments were in accordance with those of Ritzman and Levin (20) However no clinical

Book reviews

Inhaled particles and vapours. Edited by C. N. Davies. 495 pp. Price: £ 5. Pergamon Press, Oxford 1961

The British Occupational Hygiene Society organized in 1960 an International Symposium in Oxford on a theme which has grown more and more pertinent the behaviour of the lung in response to foreign substances in the atmosphere. The constantly increasing air pollution in our civilized communities, the keen interest in the question of inhaling radioactive fallout, the intrabronchial distribution and elimination of inhaled particles and vapours, all these and many other problems are the subjects of worldwide research.

The present proceedings of the symposium give us an excellent survey of most of the modern knowledge on the subject. Distinguished scientists from many countries have taken part in the symposium, which seems to be the first to be devoted to the matter concerned

Stig Björkman

Stockholm

Lehrbuch und Atlas der Laparoskopie und Leberpunktion. By H. Kalk and E. Wildhirt. 247 pp. Price DM 99 — Georg Thieme Verlag Stuttgart 1962.

The term laparoscopy was coined by the Swede H. C. Iacobaeus in 1910 and to him falls the credit that this method of examination of the abdominal viscera came to be known and practised in the Scandinavian countries at an early stage. He himself, however made no efforts to arise an extended interest in the method Only two or three short papers on the question were published by him about fifty years ago He did not know that at the beginning of the century Kelling had already tried out the method on animals and also in a few cases, on man.

Ignorant of Kelling's as well as of Iacobaeus contributions Kalk started laparoscoping in 1924 Since then he has reported on his observations and called attention to the diagnostic importance of the method in numerous publications.

The present work contains an accurate description of the technical performance of the examination a survey of the indications and contraindications for laparoscopy and of the additional diagnostic methods that laparoscopy makes possible, for instance needle biopsy of the liver splenoportography etc.

The main part of the book deals with the laparoscopical observations of normal and pathological findings in the abdomen. It is finely illustrated by many high-class drawings and photographs, most of them in colour

Indeed laparoscopy is a diagnostic method that is still too little practised. It is easy to perform and can give very valuable information. For a correct appraisal of the findings however a rather wide experience is required, as is strongly emphasized by Kalk and his co-workers.

The book is an excellent manual for those who intend to start laparoscopic examinations as a diagnostic aid or who have already acquired some experience of the method

Stig Björkman

Stockholm

on the sensitization of red cells by cold agglutinins and complement as shown by the Coombs test

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Book reviews

Inhaled particles and vapours. Edited by C. N. Davies. 495 pp. Price £ 5. Pergamon Press, Oxford 1961.

The British Occupational Hygiene Society organized in 1960 an International Symposium in Oxford on a theme which has grown more and more pertinent: the behaviour of the lung in response to foreign substances in the atmosphere. The constantly increasing air pollution in our civilized communities, the keen interest in the question of inhaling radioactive fallout, the intrabronchial distribution and elimination of inhaled particles and vapours, all these and many other problems are the subjects of worldwide research.

The present proceedings of the symposium give us an excellent survey of most of the modern knowledge on the subject. Distinguished scientists from many countries have taken part in the symposium, which seems to be the first to be devoted to the matter concerned.

Stig Björkman

Stockholm

Lehrbuch und Atlas der Laparoskopie und Leberpunktion. By H. Kalk and E. Wildhirt. 247 pp. Price DM 99. — Georg Thieme Verlag, Stuttgart 1962.

The term laparoscopy was coined by the Swede H. C. Iacobaeus in 1910, and to him falls the credit that this method of examination of the abdominal viscera came to be known and practised in the Scandinavian countries at an early stage. He himself however made no efforts to arise an extended interest in the method. Only two or three short papers on the question were published by him about fifty years ago. He did not know that at the beginning of the century Kelling had already tried out the method on animals and also in a few cases, on man.

Ignorant of Kelling's as well as of Iacobaeus' contributions Kalk started laparoscoping in 1924. Since then he has reported on his observations and called attention to the diagnostic importance of the method in numerous publications.

The present work contains an accurate description of the technical performance of the examination, a survey of the indications and contraindications for laparoscopy and of the additional diagnostic methods that laparoscopy makes possible, for instance needle biopsy of the liver, splenoportography etc.

The main part of the book deals with the laparoscopical observations of normal and pathological findings in the abdomen. It is finely illustrated by many high-class drawings and photographs, most of them in colour.

Indeed laparoscopy is a diagnostic method that is still too little practised. It is easy to perform and can give very valuable information. For a correct appraisal of the findings however a rather wide experience is required, as is strongly emphasized by Kalk and his co-workers.

The book is an excellent manual for those who intend to start laparoscopic examinations as a diagnostic aid or who have already acquired some experience of the method.

Stig Björkman

Stockholm

on the sensitization of red cells by cold agglutinins and complement as shown by the Coombs test.

Acknowledgments

We wish to thank F Neukirch, M.D Head of the Medical Department, Militærhospitalet, Copenhagen, for helpful advice and for permission to study case 2

We are indebted to Dr Alice Reyn, the Neisseria Department, Statens Seruminstitut for the complement t trations.

Grants were received from the P Carl Petersen s Fond (K. L.) and from Anders Hasselbalch s Fond t'l leukemiens bekæmpelse (H. O)

We should also like to express our gratitude to The Distillers Company London, for kindly supplying the penicillamine.

For technical assistance we are much ind bted to Miss Merete C. Bro.

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General pathology Ed. 3 Edited by Sir H. Florey 1104 pp Price: 120s. net. Lloyd-Luke Ltd., London 1962.

The new edition of Florey's *General Pathology* has increased by 72 pages, due in part to the index being twice as long A chapter has been added on immunological problems associated with transplantation of tissue, and the previous chapters have been revised. One is particularly struck by the addition of the numerous electron photograms and coloured photographs.

In the otherwise interesting and finely illustrated 30-page chapter on normal and pathological production of mucus, the reviewer has sought in vain for a single reference to mucoviscoidosis

The significance of a sudden drop in blood pressure as cause of, for example, cardiac infarction or encephalomalacia is not mentioned despite the enormous importance of this so-called Buchner mechanism — to name two shortcomings in this otherwise so readable book.

A more complete review of the Second Edition was published in this journal, Vol. CLXI fasc. VI, 1958.

Åke G H. Lindgren

Stockholm

Liver biopsy By R. G Shorter An atlas of histologic appearances. 111 pp. 107 figs. Price 60s. net. Pergamon Press Ltd., Oxford 1961

A very welcome atlas of the histologic picture of liver punctate in numerous ailments. Each section contains a brief, very personal survey of the author's own and earlier findings and is illustrated by microphotographs of high quality It is only to be regretted that at least some of the illustrations for example of biliary stasis and amyloidosis, are not in colour even if this would have added to the price of the book.

Surprisingly enough, no mention is made of schistosomiasis among the parasitic diseases despite its enormous importance in producing severe lesions of the liver in millions of people, especially in tropical countries — it must be one of the most serious diseases afflicting the human race!

Åke G H Lindgren

Stockholm

Tumors of bone and cartilage By L. V Ackerman and H. J Spjut Armed Forces Institute of Pathology Washington, D C. 1961 347 pp 362 figs. Price $3.00

Tumors of the odontogenic apparatus and jaws By J L. Bernier 107 pp., 120 figs. Price: $1 00 Ditto 1960.

Tumors of the female sex organs Part 3 By A. T Hertig and H. Gore. 178 pp., 157 figs. Price $1 40 Ditto 1961

Like all other volumes from Armed Forces Institute of Pathology these are of top class both textually and in their abundance of illustrations. The cheap prices always come as a happy surprise to the reader There can hardly be more elegant publications in existence.

Åke G. H. Lindgren

Stockholm

Lehrbuch der inneren Medizin Edited by H Dennig Vol I 996 pp Vol. II 901 pp Price DM 54 — per vol. Georg Thieme Verlag Stuttgart 1961

The 5th edition of this textbook — the 4th appeared in 1957 — has undergone *certain revisions and additions*. Thus the chapter on infectious diseases has been modernized in several respects and in a very satisfactory manner The section on diseases of the kidneys and urinary tract has been altogether re written by a new author Kleinschmidt of Mainz which must be considered a definite improvement.

As I pointed out in my review of the 4th edition a textbook which is written by a number of specialists must be of irregular quality This is true also of the present edition Here and there one finds information which is surprisingly out of date especially perhaps concerning therapy A chapter which is in great need of modernization is the section on diseases of the respiratory organs. Other chapters however are quite up to date

The rather sparse bibliographies con tain — as in the 4th edition — almost solely works of German authors and studying the textbook, one gets the impression that it is too much directed to German readers.

In spite of these critical points of view the book is on the whole to be recommended

Stig Björkman

Stockholm

Pathology of the lung (Excluding Pulmonary Tuberculosis) By H. Spencer 850 pp 503 figs. Price £10 net. Pergamon Press, Oxford London New York Paris 1962.

Not since Fischer s chapter on pulmonary diseases in Henke Lubarsch s great handbook on pathology can this theme have been so thoroughly and instructively handled as in this monography A. A. Liebow writes in the preface "Only a rare concurrence of meticulous scholar ship and discernment could have enabled the condensation of so much information into so little space. This work will long be of interest and value to all students of disease

The entire pathology of the lung with the exception of tuberculosis is dealt with in 23 chapters and illustrated by 503 extremely fine photographs, some of which in colour Some angiograms and X ray photographs relate especially to pathophysiological problems. The bibliography contains over 1 800 authors, mostly English speaking of the remainder some are German French and Scandinavian and a few from other countries.

This admirable work should be greeted with delight especially by pathologists, lung physicians and thoracic surgeons, while the chapters on fungoid infections and parasitic pulmonary ailments will be of great interest to physicians in tropical countries.

Åke G H. Lindgren

Stockholm

Acta Medica Scandinavica. Vol. 173, fasc. 6, 1963

From the Department of Medicine and the Isotope Laboratory of the St. Annadal Hospital
(Head J. M. Coenegracht, M. D.) Maastricht, Holland

Demonstration of Possible Auto-antibodies Against I.F in Pernicious Anaemia

By

J. M. Coenegracht and D. E. Mendes de Leon
with the technical assistance of Miss G. Hellwig

During the last two years an increasing number of reports concerning the role of auto-antibodies in pernicious anaemia have appeared in the literature (5, 6, 10).

At first it was presumed that only acquired resistance to oral preparations containing hog intrinsic factor (I.F.) + vit. B_{12} was based upon immunological factors, i. e. the development of heterologous antibodies against hog I.F. (4). Apart from the fact that acquired resistance to oral antigens in adult persons is rather uncommon, it soon became clear however that not only cross-resistance with human I.F. existed, but that even a certain number of sera from patients suffering from pernicious anaemia, who had received only parenteral treatment or no treatment at all, were capable of reducing the absorption of vit. B_{12} + I.F. (6, 10). Hence the hypothesis that pernicious anaemia could be considered as another example of auto-immune disease, associated with the production of auto-antibodies against gastric mucosa cells or enzymes secreted by these cells, was laid open to discussion and prompted further investigation. The results of the experiments of Taylor and Morton (9), Taylor (10) and Schwartz (5, 6) in particular support this new concept of the pathogenesis of pernicious anaemia. Several controversial points in this field however are not yet elucidated. One is, indeed struck by the unexplained fact that in only a limited number of sera from patients with pernicious anaemia can neutralizing antibodies be demonstrated, other samples failing to exhibit these properties. Th[e] question therefore arose whether the quantitative relationship between antigen and antibody, the temperature and time of incubation and the influence of the pH played a role in the outcome of the eventual immune-reaction involved.

In the present study we shall first record the results of long-term treatment with an oral preparation in 10 patients

Submitted for publication November 20, 1962.
43—633003 Acta Med. Scand. Vol. 173

Early detection and diagnosis of cancer By W E. O Donnell E Day and L. Venet 286 pp 82 fig Price $ 12 00 The C. V Mosby Company St. Louis Miss. 1962

One hundred years ago a patient with cancer had little chance of survival. To-day according to statistics a cancer patient has one chance in three of getting cured This improvement in prognosis is primarily due to better surgical and radiological treatment but undoubtedly diagnosing the disease at the earliest possible stage is of considerable importance. It has been estimated that to-day half of all cancer patients could be cured if the lesions were detected as early as is now possible So there is still a gap between the third of the cancer patients cured and a potential cure rate of 50 per cent

One can agree with the authors of this book that a realistic program of cancer control must start with the family physician who is often the first to see the patient On him rests the responsibility of how long a time elapses from symptoms to final diagnosis and adequate treatment.

The three members of the staff of the American Sloan Kettering Cancer Institute describe the methods available for early detection and diagnosis of cancer how to look for and diagnose cancer in the physician's office. The text is condensed but well written and numerous drawings and other visual aids facilitate its understanding Treatment and prognosis of cancer are not dealt with and there is no bibliography The authors are convinced — and they are surely right — that lives at present lost could be saved through the application of the techniques of cancer detection diagnosis, and prevention described in this book.

Johannes Mosbech

Copenhagen

Praktische Gastroenterologie 2 edition. By Ernst Hafter 478 pp. 169 fig Price D M. 59 — Georg Thieme Verlag, Stuttgart 1962

The author who is attached to the University Clinic of Zürich as a lecturer in gastroenterology has had the satisfaction to see a second edition of his book brought out after only six years, and in the meantime Polish Spanish and Italian translations have appeared

After introductory chapters on general diagnostics and therapy the book contains sections dealing with the diseases of the different parts of the gastrointestinal tract, including the liver and pancreas. The enormous amount of material is clearly arranged and the judgements generally appear well balanced. The bibliography includes 1,250 references. The book deserves the wide distribution already achieved

Johannes Mosbech

Copenhagen

Acta Medica Scandinavica, Vol. 173, fasc. 6, 1963

From the Department of Medicine and the Isotope Laboratory of the St. Annadal Hospital
(Head J. M. Coenegracht, M. D.) Maastricht, Holland

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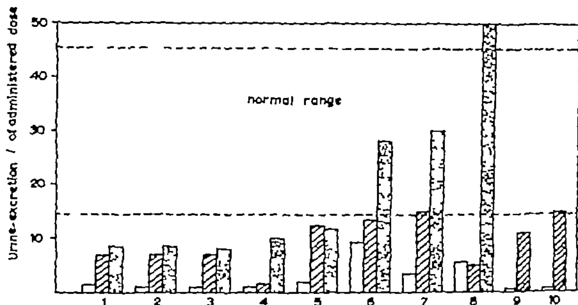


Fig. 1 Patients, suffering from pern. anaemia after prolonged treatment with bifacton.
Schilling test without addition of hog I.F or human I.F
Schilling test with addition of hog I.F
Schilling test with addition of human I.F

suffering from pernicious anaemia and then compare the inhibitory properties of the sera from some of these patients, who had developed a certain degree of resistance to hog I F and human I.F with sera from patients not receiving oral therapy Finally we have investigated in a few cases the influence of the pH during the incubation period

Material and methods

All patients were suffering from classical pernicious anaemia patients with steatorrhoea, carcinoma or resection of the stomach were excluded from this study The renal functions were not investigated in detail none of the patients, however had clearcut signs of renal insufficiency

Oral treatment consisted of 2 or 3 tablets of bifacton (Organon) daily Bifacton contains 7.5 γ of vit. B_{12} and 20—25 mg of purified hog I.F per tablet.

The serum level of the vit B_{12} was determined on two occasions at the end of the oral months, before the Schilling-tests were carried out[1]

The Schilling-tests were performed with an oral dose of 0.5 γ of vit. B_{12} containing 1 μC of Co^{60} with a half life of 71 days after 2 hours and 24 hours respectively the patients received an i.m. injection of 1 mg of vit. B_{12}. The excretion of Co^{60} in the 0—24 and 24—48 hours urine was measured directly in a well type scintillation counter equipped with a pulse-height analyser Duplicate samples of 2 ml of urine were tested for a total of 5,000 counts. The statistical counting error was ± 2 for specimens of high activity and ± 6 for specimens of low activity In normal persons we found the same values as other investigators (12—46 (1))

The tests were repeated with addition of hog pyloric mucosa, containing 0.5 U.S.P (about 20—25 mg) of purified hog I.F — unless otherwise stated. Human gastric juice (70 ml) containing free hydrochloric acid and stored

[1] These determinations were carried out in the laboratories of Organon, Oss, by means of the Lactobacillus Leichmanii method. We are also indebted to Organon for providing us with hog ... large quantities of vit. B_{12}

at 4° C for not longer than a few days was used as source of human LF.

In the neutralization experiments the incubation of serum (40 ml) and LF took place at room temperature (± 20° C) for about 20 minutes, then the mixture was given to the patients together with the labelled vit. B_{12}. No correction was made for the pH, unless otherwise stated.

The interval between the Schilling-tests was at least 4 days.

Results

A. The results of oral treatment lasting 21—64 months in 10 patients are summarized in table I and fig. 1. None of the patients showed a pronounced haematological relapse, although the number of erythrocytes was somewhat lowered in several cases, with a minimum of 3.33 million in the last patient (no. 10); this 75-year-old man was also suffering from a chronic lung disease (tbc). Otherwise the fluctuations of haemoglobin content and erythrocyte counts, reckoning from the moment that remission was achieved, remained within normal or eventually low normal limits. All patients felt well on oral therapy with the exception of case no. 8, who complained of fatigue and dizziness at the end of the observation period. This man, too, showed mild symptoms of neurological involvement; his serum level of vit. B_{12} was amongst the lowest observed, namely 70 $\mu\gamma$/ml.

The *serum level of vit. B_{12}* at the end of the observation period varied from 60 to 360 $\mu\gamma$/ml with a mean value of 136 $\mu\gamma$/ml. Only two patients showed at both determinations a normal serum level of vit. B_{12}, that is to say according to Killander (3) and Spray and Witts (7) above 150 $\mu\gamma$/ml.

In two other patients the vit. B_{12} values were only slightly reduced and fluctuated between 100 and 200 $\mu\gamma$/ml.

Table I. Patients suffering from pernicious anaemia after prolonged treatment with bifacton

Pat.	Age (yrs)	Sex	Duration of bifacton therapy (months)	Serum Vit. B_{12} ($\mu\gamma$/ml)	Serum Iron (γ %)	Schilling test %
1	67	o	30	94 85	143	1.5 b 7.2 c 8.2
2	67	o	21	170 60	110	a 1.0 b 7.0 c 8.2
3	63	♀	38	150 185	110	0.9 b 7.3 c 6.7
4	62	♀	31	200 150	110	1.0 b 1.1 c 10.0
5	76	♀	33	80 85	87	1.8 b 12.7 c 12.0
6	45	o	64	360 240	112	9.9 b 15.5 c 28.0
7	70	♂	42	80 120	164	a 3.1 b 15.2 c 50.4
8	58	o	40	70	114	a 5.9 b 5.5 56.5
9	71	♀	35	170 175	125	0.2 b 11.3
10	75	♂	24	75 80	35	0.7 b 14.1

a) without addition of hog LF or human LF; b) with addition of hog LF and c) with addition of human LF

The remaining 6 patients had at least on one occasion a definitely lowered serum level of vit. B_{12}.

No relationship was found between the duration of oral therapy and the serum content of vit. B_{12}.

Table II Results of neutralization experiments with sera from patients treated with bifacton

Pat. no.	Age (yrs)	Sex	Therapy	Schilling test (%)	Serum donor
1	72	♂	Vit B_1 i.m. (8 mg)	a 0 0 b 12.6 b 13 7 c 24.2 c 3 7	Pat. no. 2 (table I)
	42	○	Vit. B_1 i. m. (12 mg)	a 0 0 b 16.3 b 14 6 c 43.2 c 11.6	Item
3	51	♂	Vit. B_1 i. m. (14 mg) Bifacton since 8 months	a 0.8 b 13.4 b 8.5 c 26.0 c 6 6	Pat. no. 1 (table I)
4	45	♀	Vit. B_{12} i. m. (12 mg). Bifacton since 6 months	a 0 0 b 13 4 b 41 3 c 62.5 c 14.8	Pat. no. 3 (table I)

[1] a) without addition of hog I F or human I.F., b) with addition of hog I.F b) with addition of "neutralized hog I.F., c) with addition of human I.F c) with addition of "neutralized human I.F

The *serum iron* content of all patients was within the normal range, except in the last case where the picture was complicated by chronic lung disease.

The *Schilling-test*, performed without addition of I F showed as expected a very low urinary excretion of vit. B_{12}. However case no 6 was an exception and excreted as much as 9 9 % (11 4 % at a control a few months later) of the administered dose, in spite of the fact that he presented at the beginning of his disease all the classical signs of pernicious anaemia including a typical megaloblastic bone marrow and a pronounced reticulocyte response to vit. B_{12}. It is perhaps of interest to note that this patient was the only one who was treated right from the beginning exclusively with bifacton and, moreover was the only one who neglected repeatedly to take the daily dose of bifacton

Addition of purified hog stomach to the oral dose of vit. B_{12} restored the excretion in the 48-hour urine to slightly subnormal values in 5 patients (nos. 5 6 7 9 10) produced only a small increase in 3 other patients (nos. 1 2 3) and had no appreciable effect in the remaining two (nos. 4 8)

Without excluding the possibility that the employed dose of hog I.F was too low to obtain a maximum resorption of vit. B_{12}, we still observed that 3 of the 5 patients who failed to respond sufficiently on addition of hog stomach powder appeared to be equally unresponsive when human gastric juice was employed as a source of I F (nos. 1 2, 3)

We could not establish any relationship between the duration of oral therapy or the serum level of vit. B_{12} on one hand and the type of reaction to the various modifications of the Schilling test on the other

In general, our clinical and haematological results appear somewhat more favourable than those of other investigators whereas we could confirm the occurrence of low values of serum vit. B_1 on prolonged oral treatment.

Although the number of our cases is limited we are inclined to the conclusion that the impaired absorption of vit. B_{12} cannot be ascribed to the development of refractoriness to hog I.F alone, and that responsiveness cannot always be restored by addition of human gastric juice.

B Sera from three of these patients (nos. 1 2 3) were tested for inhibitory

action upon hog and human I.F These sera were mixed with hog stomach powder or human gastric juice and then administered, together with the test dose of radioactive vit. B_{12}, to patients also suffering from pernicious anaemia but who had never received oral therapy (table II).

None of the latter showed impaired resorption of vit. B_{12} + hog I.F or human I.F without the addition of the sera to be tested. The results of these experiments suggest the presence of neutralizing antibodies against human I.F rather than against hog I.F the activity of which appeared not to be markedly reduced in one case (no. 4) even raised

C. Finally we tried to demonstrate antibodies against hog I.F and human I.F in sera from patients who had received no oral therapy (In these experiments the double dose of hog I F was used our previous observations had left us with the impression that a maximum response was not obtained with the initially employed dose of 20—25 mg) The results are summarized in table III. The majority of the investigated sera had no influence on the activity of hog I.F (inhibition absent in 6 cases — 2b 3b 4b 4b 5b 5b — present in only one case, no. 1b') nor did they block the activity of human I.F (inhibition absent in 5 cases — 3c 4c 4c 5c 5c — present in only one case no. 1c') It should be mentioned however that in one or two cases in which the demonstration of antibodies had been previously unsuccessful, a positive result was obtained when the pH of the mixture of serum + human gastric juice was adjusted to 7 by addition of 0 1 N NaOH (patient no. 4c and possibly also patient no. 5c) Alteration of the pH as such did not reduce the absorption of vit. B_{12} +

Table III Results of neutralization experiments with sera from patients not receiving oral therapy

Pat. no.	Age	Sex	Therapy	Schilling test %
1	58	♂	Vit. B_{12} i. m. (5 mg)	a 9.0
				b 21.6
				b 8.0
				14.6
				6.5
2	71	♀	Vit. B_{12} i. m. (5 mg)	0.0
				b 20.6
				20.0
3	49	♀	—	5.1
				b 29.5
				b 23.5
				11.6
				~ 12.5
4	74	♀	—	4 1
				b 25.9
				b 34.1
				b~ 39.5
				20.4
				~ 33.2
				33.2
				12.3
				~ 35.8
5	64	♂	—	0.6
				b 13 0
				b 17.3
				b 25.3
				13.4
				15.0
				23.1
				~ 13.0

) without addition of hog I.F or human I.F b) with addition of hog I.F b') with addition of "neutralized" hog I.F b) with addition of "neutralized" hog I.F (own serum)) with addition of human I.F ') with addition of "neutralized" human I.F and) with addition of "neutralized" human I.F (own serum)
 incubation at pH 7

human I.F., at least in the one case investigated (patient no. 4c)

Remarkable in this respect are the following observations

Table II Results of neutralization experiments with sera from patients treated with bifacton

Pat. no.	Age (yrs)	Sex	Therapy	Schilling test (%)	Serum donor
1	72	♂	Vit. B_1 i.m. (8 mg)	a 0.0 b 12.6 b 13.7 c 24.2 c 3 7	Pat. no. 2 (table I)
2	42	o	Vit. B i. m. (12 mg)	a 0.0 b 16.3 b 14.6 c 43.2 c 11.6	Item
3	51	♂	Vit. B_1 i. m. (14 mg). Bifacton since 8 months	a 0.8 b 13 4 b 8.5 c 26 0 c 6 6	Pat. no. 1 (table I)
4	45	♀	Vit. B_1 i. m. (12 mg). Bifacton since 6 months	a 0.0 b 13 4 b 41.3 c 62.5 c 14.8	Pat. no. 3 (table I)

a) without addition of hog I F or human I.F., b) with addition of hog I.F b) with addition of "neutralized" hog I F., c) with addition of human I.F., c) with addition of "neutralized" human I.F

The *serum iron* content of all patients was within the normal range, except in the last case where the picture was complicated by chronic lung disease.

The *Schilling-test* performed without addition of I F showed as expected a very low urinary excretion of vit. B_{12}. However case no 6 was an exception and excreted as much as 9 9 % (11 4 % at a control a few months later) of the administered dose, in spite of the fact that he presented at the beginning of his disease, all the classical signs of pernicious anaemia including a typical megaloblastic bone marrow and a pronounced reticulocyte response to vit. B_{12}. It is perhaps of interest to note that this patient was the *only one* who was treated right from the beginning exclusively with bifacton and, moreover was *the only* one who neglected repeatedly to take the daily dose of bifacton.

Addition of purified hog stomach to the oral dose of vit. B_{12} restored the excretion in the 48-hour urine to slightly subnormal values in 5 patients (nos. 5 6 7 9 10) produced only a small increase in 3 other patients (nos. 1 2 3) and had no appreciable effect in the remaining two (nos. 4 8)

Without excluding the possibility that the employed dose of hog I.F was too low to obtain a maximum resorption of vit. B_{12}, we still observed that 3 of the 5 *patients who failed to respond sufficiently* on addition of hog stomach powder appeared to be equally unresponsive when human gastric juice was employed as a source of I F (nos. 1 2 3)

We could not establish any relationship between the duration of oral therapy or the serum level of vit. B_1 on one hand and the type of reaction to the various modifications of the Schilling test on the other

In general our clinical and haematological results appear somewhat more favourable than those of other investigators, whereas we could confirm the occurrence of low values of serum vit. B_{12} on prolonged oral treatment.

Although the number of our cases is limited we are inclined to the conclusion that the impaired absorption of vit. B_{12} cannot be ascribed to the development of refractoriness to hog I.F alone, and that responsiveness cannot *always be restored* by addition of human gastric juice.

B Sera from three of these patients (nos. *1 2, 3*) were tested for inhibitory

action upon hog and human I.F These sera were mixed with hog stomach powder or human gastric juice and then administered together with the test dose of radioactive vit. B_{12}, to patients also suffering from pernicious anaemia but who had never received oral therapy (table II)

None of the latter showed impaired resorption of vit. B_{12} + hog I.F or human I.F without the addition of the sera to be tested. The results of these experiments suggest the presence of neutralizing antibodies against human I.F rather than against hog I.F., the activity of which appeared not to be markedly reduced, in one case (no. 4) even raised.

C. Finally we tried to demonstrate antibodies against hog I.F and human I.F in sera from patients who had received no oral therapy (In these experiments the double dose of hog I.F was used our previous observations had left us with the impression that a maximum response was not obtained with the initially employed dose of 20—25 mg) The results are summarized in table III. The majority of the investigated sera had no influence on the activity of hog I.F (inhibition absent in 6 cases — 2b 3b 4b 4b 5b 5b — present in only one case, no. 1b) nor did they block the activity of human I.F (inhibition absent in 5 cases — 3c 4c 4c 5c 5c — present in only one case, no. 1) It should be mentioned however that in one or two cases in which the demonstration of antibodies had been previously unsuccessful, a positive result was obtained when the pH of the mixture of serum + human gastric juice was adjusted to 7 by addition of 0 1 N NaOH (patient no. 4c nd possibly also patient no. 5c) Alteration of the pH as such did not reduce the absorption of vit. B_{12} + human I.F at least in the one case investigated (patient no. 4c)

Remarkable in this respect are the following observations

Table III Results of neutralization experiments with sera from patients not receiving oral therapy

Pat. no.	Age	Sex	Therapy	Schilling test %
1	58	♂	Vit. B_{12} i. m. (5 mg)	a 9.0 b 21.6 b 8.0 14.6 6.5
2	71	♀	Vit. B_{12} i. m. (5 mg)	0.0 b 20.6 20.0
3	49	♀	—	5.1 b 29.5 b 23.5 11.6 ″ 12.5
4	74	♀	—	4.1 b 25.9 b 34.1 b″ 35.5 20.4 33.2 33.2 12.3 ″ 35.8
5	64	♂	—	0.6 b 13.0 b 17.3 b 25.3 13.4 15.0 ″ 23.1 43.0

) without addition of hog I.F or human I.F.; b) with addition of hog I.F b′) with addition of "neutralized" hog I.F b″) with addition of neutralized hog I.F (own serum) c) with addition of human I.F) with addition of "neutralized human I.F and) with addition of "neutralized" human I.F (own serum)

Incubation at pH 7

1. Serum (50 ml per os) with a known inhibitory effect upon I.F. — tested on a patient with pernicious anaemia — failed to reduce the absorption of labelled vit. B_{12} in a healthy man (9, 10).

2. Schwartz (6), using an oral dose of 10 ml of serum with I.F. antibodies, arrived at the same result in a normal person with free hydrochloric acid. However, in another subject with achlorhydria but a normal absorption of vit. B_{12}, the same amount of inhibitory serum blocked almost completely the absorption of labelled vit. B_{12}, even after the addition of hog I.F.

These facts and the negative results of our experiments can be explained in two ways:

a. Destruction of the inhibitory serum factor by acid (HCl) and proteolytic enzymes.

b. pH sensitivity of the eventually involved immune reaction.

Of course it is questionable whether this limited number of examinations justifies anything more than a tentative suggestion. It would be interesting, however, to extend these studies in order to establish the influence of the pH and eventually of other environmental factors, such as incubation time, temperature, the role of complement etc. upon the reaction between intrinsic factor and the alleged antibodies — or whatever the nature of the inhibitory substances may be — in sera from patients with pernicious anaemia. Meanwhile the possibility that humoral auto-antibodies are merely a by-product in the pathogenetic mechanism of pernicious anemia and not the cause of the disease should be kept in mind.

The recent advances in serology carry some danger of diverting our attention from the eventual role of the less easily detected process of cellular hypersensitivity or other noxious agents.

Summary

Ten patients, suffering from pernicious anaemia, did not show definite signs of clinical or haematological relapse after long term treatment with oral therapy despite an impaired resorption of vit. B_{12} + hog I.F. or human I.F. in about half the cases. Sera from these patients inhibited the activity of human I.F. but not that of hog I.F. The majority of sera from patients with pernicious anaemia not treated with oral therapy failed to reduce I.F. activity of hog pyloric mucosa or human gastric juice, but in one or two instances inhibition could be demonstrated when the pH during incubation was raised to 7. Further study is needed to elucidate the possible influence of pH and other environmental factors upon the reaction between intrinsic factor and neutralizing antibodies.

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The Control of Phenylindanedione Treatment

By

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Many modifications of the Quick test for controlling the coagulability of blood during treatment with dicoumarol-type anticoagulants have been proposed lately. The aim of these modifications has been, on the one hand, to improve the test so that it reveals better a real decrease in the coagulability and any danger of bleeding and, on the other hand, to simplify the required equipment and to render the test less time-consuming.

Previously when it was believed that the original Quick test gave only the content of prothrombin, which is only one of the dicoumarol-sensitive coagulation factors, the improvements were aimed at making the test more sensitive to factor VII (32). Later when it was found that factor VII has no influence on the intrinsic blood coagulability attempts were made to lower the sensitivity of the test to factor VII and to increase its sensitivity to factors IX and X, whose levels also vary during the anticoagulant treatment (10 38). It may be mentioned that Waaler (45) found that Owren's P & P method is likewise sensitive to either or both of the latter factors.

For a more rapid performance of the test the thromboplastin and calcium have been combined in the same reagent, the test has been applied to uncentrifuged whole blood and the blood sample has been taken from the fingertip to avoid the cumbersome venipuncture (11 17 20, 21 27 44).

It has also been proposed that the heparin-retarded clotting time (47) or a modification of it (40) or the clotting time with viper venom and cephalin (12) be used instead of the Quick test to control anticoagulant treatment, but neither of these methods has apparently found wider acceptance.

Material and methods

The purpose of the present investigation was to compare the Quick values obtained with different thromboplastin preparations, thrombo-test values, the changes in certain coagulation factors, and heparin-retarded clotting times during and after phenylindanedione treatment. In this paper the values obtained in the Quick test and its modifications are called Quick values as recommended by

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Jürgens and Beller (10) Other names such as the thromboplastin time, the prothrombin percent, etc., have been avoided as they are easily misunderstood or essentially incorrect.

The Quick values were determined during the first month of treatment with phenyl indanedione (Trombosol Oy Star Ab, Tampere, Finland) for 77 patients from whom 365 venous blood samples were withdrawn and tested employing Idokinas (Ferrosan AB Malmö, Sweden) and Thrombokinase Geigy (referred to as Geigykinase below) (J R. Geigy AG Basel, Switzerland) and for 54 patients from whose fingertips 100 blood samples were withdrawn and tested both by the thrombotest (thrombo-test value) (Oy Medica Ab, Helsinki Finland) and with Geigykinase by the micro method. Venous blood samples from 45 other patients were tested by the standard method employing the thrombo-test, and samples from the finger tips of these patients by the thrombo-test micro method. Venous blood samples and fingertip samples from a fourth group of 24 patients were tested employing Geigykinase.

In addition, the Quick values of venous blood taken from 11 patients both during and after a rather short treatment with phenyl indanedione were determined simultaneously employing Idokinas, Geigykinase, Thrombotest (thrombo-test value) and Simplastin (Warner & Chilcott, Morris Plains, N J., U.S.A.) The heparin-retarded clotting times and the prothrombin, factor VII (proconvertin) and factor X contents were also determined. All the patients were under observation before the treatment and during the period of treatment which lasted from 4 to 20 days (10 days on the average) and nine patients in addition during 4—10 days (6 days on the average) after the treatment was discontinued.

The coagulability of the blood of 28 patients who had been under phenylindanedione treatment for several months was also studied employing the same tests.

Each Quick value determined with Idokinas is in this paper given as an index computed by dividing the Quick time of normal blood by the Quick time of the patient's blood and multiplying the result by one hundred this is a common practice in this country The result could, of course, have been reported as a percentage, but would then have been difficult to interpret because the calibration might have been done with normal plasma that had been diluted with physiological saline (34a) a fibrinogen solution (15) a fresh adsorbed plasma (32) or an aged adsorbed plasma (10) The Quick values determined with Geigykinase and Simplastin and the thrombo-test values are given as percentages read from curves given by the manufacturers it was confirmed that the Quick times (thrombo-test times) for normal plasma were the same as those given by the manufacturers.

A Veronal-buffered, isotonic sodium chloride solution of pH 7.35 was used as diluent in all the tests. The blood was mixed with 3 8 % sodium citrate solution in the ratio of 1 10 or 1 5 as recommended for the thromboplastin preparation in question by the manufacturer

The heparin-retarded clotting times (HRCT) were determined by the method of Waugh and Ruddick (47) One millilitre of the patient's blood was directly added without using a syringe to a test tube containing 0.5 ml of the isotonic sodium chloride solution and 0.5 unit of heparin (Heparin Medica Inject Oy Medica Ab Helsinki) The test tube was closed with a rubber stopper inverted twice to mix the blood with the heparin solution and left to stand at room temperature The actual clotting time was recorded when the first signs of clotting were noted on tilting the test tube.

The prothrombin free substrate was prepared by mixing two parts of adsorbed bovine plasma and one part of human serum. For the determination of prothrombin content 0.1 ml of a 1 10 dilution of the plasma was added to 0 1 ml of the substrate and the resulting mixture heated half a minute at 37° C, after which 0.2 ml of Geigykinase was added to it.

The substrate free from factor VII and the cephalin which was needed in the tests for factor X were obtained from the Central Laboratory of Finland Red Cross Blood Service. The substrate free from factor VII had been prepared in the usual manner by filtering bovine plasma through an asbestos filter and the cephalin had been isolated from human brain tissue by acetone-ether extraction. Stypven (Burroughs, Wellcome & Co., London) was used as the viper venom source in the determination of factor X.

The factor VII content was determined similarly as the prothrombin content, but using substrate free from factor VII instead of one free from prothrombin.

For the determination of factor X, 0.5 ml of Stypven diluted with distilled water was thoroughly mixed with 9.5 ml of cephalin diluted 1 300 with buffered saline 0.1 ml of this mixture, 0.1 ml of the factor VII-free substrate and 0.1 ml of the 1 10 dilution of the plasma under study were added to a test tube, mixed and heated half a minute at 37° C, after which 0.1 ml of 0.025 molar calcium chloride solution was added to the mixture.

The recorded clotting times were compared with curves for normal plasma diluted with buffered saline.

The actual investigation proceeded as follows. Blood collected by venipuncture for determining the Quick values and the coagulation factors was taken into one test tube containing one part of sodium citrate to nine parts of the blood and into second test tube containing one part of sodium citrate to four parts of the blood. Tw additional samples of the blood ere taken into test tubes for study ing the HRCT The HRCT and the Idokinas, Geigykinase and Thrombotest values were then determined for the whole blood. The Simplastin value was determined for plasma obtained by centrifugation of the blood. The remaining plasma (citrated 1 10) was frozen in siliconized test tubes and stored at — 20° C. All the blood samples collected from a patient were examined at one time for coagulation factor levels.

All the tests were run t least in duplicate.

Results

The results obtained when venous blood samples were tested with Idokinas and Geigykinase are shown in figs 1 –3 "Therapeutic Idokinas indexes from 30 to 60 correspond to Quick percentages from about 10 to 30 (as determined by the method of Quick (34a)) As the "therapeutic" range of Quick alues obtained with Geigykinase is 15—25 ac cording to Montigel (22) and 18—30 %

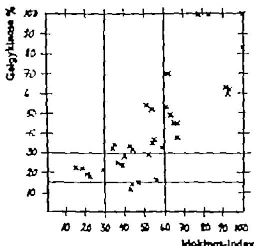


Fig. 1 Quick values obtained with Idokinas (ex pressed as indexes) and with Geigykinase (expressed in %) on the first three days of phenylindanedione treatment. Each cross represents one blood sample.

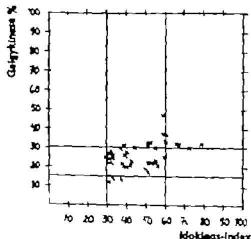


Fig. 2 Quick values obtained with Idokinas and Geigykinase on the fourth to seventh days f treat ment.

according to Schlegel and Montigel (36) the range has been taken to be 15—30 Fig 1 shows that both these thromboplastins gave the same results (the Quick value indicated a risk of bleeding was

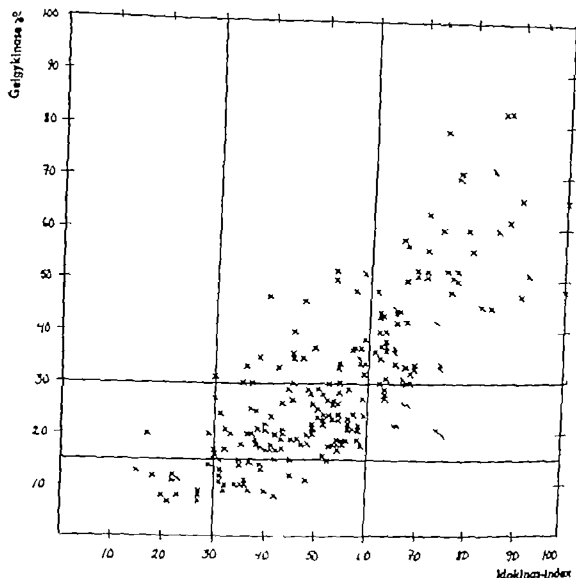


Fig 3 Quick values obtained with Idokinas and Geigykinase in the second to fourth weeks of treatment.

at the therapeutic level, or showed an underdosage) in 34 cases out of 59 Idokinas indicated the treatment to have been *more effective than did Geigykinase* in 22 of the remaining cases (37 %) and less effective in 3 cases (5 %)

Fig 2 shows that in the second half of the first week both thromboplastins gave the same results in 40 cases out of 62 whereas Idokinas showed the treatment to be more effective than did Geigykinase in 16 cases (26 %) and less effective in 6 cases (10 %)

Fig 3 shows that when the treatment had continued more than one week but *less than one month, these two thromboplastins gave identical results in 196 cases out of 276* whereas Idokinas showed the treatment to be more effective than Geigykinase did in 35 cases (13 %) and less effective in 46 cases (17 %) Because of the rather large individual variations (fig 3) two lots of Idokinas and Geigykinase were tested separately against each other The differences due to experimental variations were found to be very

small and hence the deviations in fig 3 were concluded to be most probably due to the fact that the two thromboplastin preparations react partly with different coagulation factors.

From the data in these three figures it may be concluded that Idokinas showed the treatment to be more effective than did Geigykinase on the first three days of treatment. In the second half of the first week the differences revealed by the two thromboplastins diminished, and from the beginning of the second week Idokinas tended to show the treatment to be less effective than Geigykinase did.

Fig. 4 shows that parallel determinations of Quick values with Geigykinase and thrombo-test values gave the same results for 17 of 42 samples in the first week. The therapeutic range reported by the manufacturer for Thrombotest is 10—25 %. In the other 25 cases (60 %) Thrombotest revealed the treatment to be more effective than did Geigykinase. The results were similar in the first and second half of the first week. Fig 5 shows that the situation remained about the same for the rest of the first month. Thrombotest showed the treatment to be more effective than did Geigykinase in 23 cases (66) out of 35 Thrombotest thus showed the treatment to be more effectiv than did Geigykinase during the whole of the first month.

These differences were more pronounced in the cases where the dosage was revealed to be sufficient by t least one of the two thromboplastins under comparison. Idokinas showed the treat ment to be more effectiv than did Geigykinase in 63 of the cases during the first three days and in 30 of the cases during the following four days, and Thrombotest showed the treatment to be more intense than did Geigykinase in 86 / of the cases during the first week and in 76 % of the cases subsequently

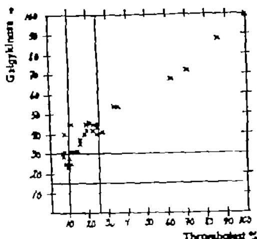


Fig. 4 Thrombotest alues (in %) and Quick values obtained with Geigykinase in the first week of treatmen

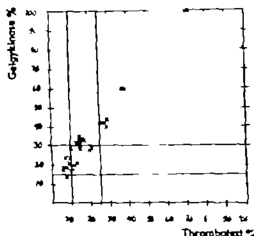


Fig. 5 Thrombotest values and Quick values obtained with Geigykinase in the second to fourth weeks of treatment.

When blood from 45 patients was tested by the standard method (venipuncture) and by the micro method (from the fingertip) sim ltaneously employi g thrombotest, the values showed

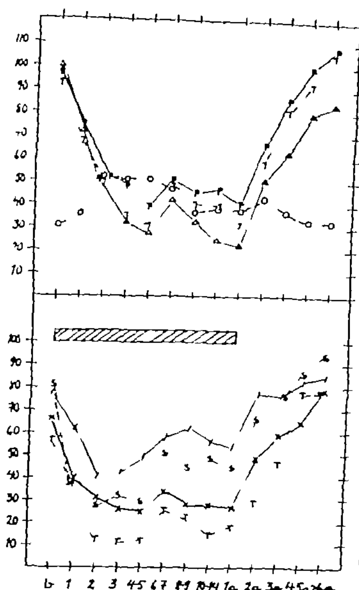


Fig 6 Variations in Quick values and thrombotest values obtained with different thromboplastin preparations and simultaneous variations of the HRCT and prothrombin, factor VII and factor X contents in 11 patients during and after a short phenylindanedione treatment.
Δ = factor X ( ) 7 = factor VII (%) P = prothrombin (%) o = HRCT (mm.) / = Idokinas (index) x = Geigykinase (%) S = Simplastin (%) T = Thrombotest (%)
Symbols below the figure denote the following b = before treatment, 1—14 = days of treatment, 1a—6a = days after termination of treatment.

a mean deviation of 6 per cent A similar study of blood from 24 patients employing Geigykinase gave values showing also a mean deviation of 6 per cent. The observed deviations were random. The micro methods were very simple to carry out were easily learned and saved time

Fig 6 shows the mean Quick values (thrombo-test values) obtained using different thromboplastin preparations for 11 patients during and after a short phenylindanedione treatment. During the first three days of treatment the thrombo-test values decreased close to the lower limit of the therapeutic range, the Idokinas values to the middle of the range and the Geigykinase and Simplastin values to the upper limit of the range (10—30 % for Simplastin) On the 4th—14th days of the treatment the mean thrombo-test values were in the middle of the therapeutic range the mean Quick values obtained with Idokinas and Geigykinase at the upper limit and the values obtained with Simplastin definitely above the range. The values for the second week were

notably higher than the values for the first week owing to the higher dosage in the first week. The results obtained with Geigykinase revealed this difference less clearly than those obtained with the other thromboplastin preparations.

Beginning on the first day after the termination of the treatment the values began to rise at approximately the same rate, the thrombo-test values most rapidly and the Geigykinase values least rapidly.

A very good correlation is revealed by the HRCT and Quick values as the latter decrease, the HRCT's become longer.

No striking differences were noted when the changes in the levels of different coagulation factors were examined. The prothrombin level, however, tended to decrease rather more slowly at first than the levels of factors VII and X. The level of factor X, however, continued to decrease until the therapy was terminated whereas the factor VII and prothrombin levels decreased to a minimum on the fourth or fifth day of treatment. None of the factors examined decreased to very low levels during the treatment. This is in accord with the observation that most of the thromboplastins used gave Quick values that were at the upper limit or above the therapeutic range. When the therapy ended, the prothrombin and factor VII levels rapidly reverted to their original values and even exceeded 100 % one week later whereas the factor X level rose more slowly and remained below 100 % over the whole of the observation period.

When the curves for all the 11 patients were compared differences between different days and different patients were naturally evident. On the other hand, the values obtained with the different thromboplastin preparations and the levels of the various coagulation factors varied quite uniformly in all the patients.

When the Quick values of blood samples of 28 patients who had been under phenylindanedione treatment for several months were determined with Simplastin, the values for 15 patients showed an underdosage and those for 13 patients were at the therapeutic level. Idokinas gave similar results underdosage for 12 and therapeutic levels for 16 patients. Geigykinase revealed underdosages in 7 patients, therapeutic levels in 10 patients, and a danger of bleeding in 11 patients. Thrombotest revealed underdosages in 2 therapeutic levels in 13 and a danger of bleeding in 13 patients.

The HRCT exceeded 40 min. in 3 of 14 patients for whom most of the thromboplastin preparations showed an underdosage and in 9 of 14 patients whose Quick values suggested a danger of bleeding.

The prothrombin levels varied from 13 to 95 % (mean 33 %) the factor VII levels from 5 to 50 % (mean 16) and the factor X levels from 6 to 50 (mean 20)

The results obtained with the different thromboplastin preparations after a longer period of treatment varied as much as those obtained during the first month of treatment. The one change observed was that Idokinas showed the dosage to be considerably less effective than did Geigykinase which in turn showed the dosage to be almost as effective as did Thrombotest.

As expected, the HRCT was longer in the patients who had low Quick values. Because the levels of the different coagulation factors decreased in about the same proportions in all the patients, no definite differences became evident in the reactions of different thromboplastin preparations to them.

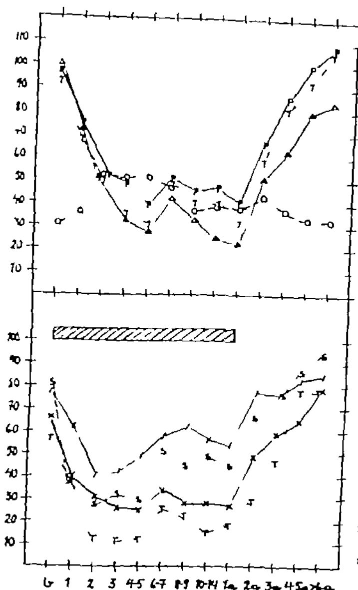


Fig 6. Variations in Quick values and thrombotest values obtained with different thromboplastin preparations and simultaneous variations of the HRCT and prothrombin, factor VII and factor X contents in 11 patients during and after a short phenylindanedione treatment.
Δ = factor X ( ) 7 = factor VII (%), P = prothrombin (%) o = HRCT (min.) / = Idokinas (index) x = Geigykinase ( ) S = Simplastin ( ) T = Thrombotest ( )
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therapeutic results presented in papers on anticoagulant treatment.

The HRCT was found in this study to be highly correlated with the Quick value. Gormsen (5) however found a lack of correspondence between these two tests, especially during the first two weeks of treatment. It has also been established that the HRCT varies after coronary thrombosis even when anticoagulant has not been administered and often in the same way as observed in this study during anticoagulant treatment (3, 5, 6, 26, 33 50). The normal variation of the HRCT is, moreover rather large and even during the anticoagulant treatment it did not rise to levels found in other patients who had not received anticoagulants (5a). Such being the case, this test evidently is not suitable for the control of anticoagulant treatment, not in its present form at least.

The results of the study of the coagulation factor levels are in qualitative agreement with the picture given in the literature: factor VII decreases first, then prothrombin and factor X. That the differences between them did not show up as clearly as has been reported previously may have been partly due to the fact that it has not been possible to differentiate the factors completely so that, for example, the variations in the prothrombin content may have to some extent been reflected in the results of the factor VII and factor X determinations.

Compared with values obtained with other thromboplastin preparations the values obtained with Geigykinase are on a higher level during the first week of treatment than during the second week. This is in accord with the generally accepted view which is also supported by the present data, that the decrease in factor VII content is the main reason for the decline in the Quick value in the early stages of anticoagulant treatment. The decreases in the prothrombin and factor X levels at later stages of the treatment have a more pronounced effect on the Quick values. Also the fact that Geigykinase showed the treatment to be more effective than, for example, did Idokinas after a longer period of treatment indicates the same.

Summary

Quick values have been determined with three different thromboplastin preparations, Idokinas, Thrombokinase Geigy (Geigykinase) and Simplastin, and thrombo-test values with Thrombotest during anticoagulant treatment with phenylindanedione. Also the heparin-retarded clotting times (HRCT) and the prothrombin, factor VII and factor X levels were followed.

A simultaneous study of blood samples with Idokinas and Geigykinase revealed that the former showed the treatment to be more effective than did the latter on the first days of treatment, but the difference gradually disappeared during the rest of the first week and the first month, and became the opposite after the first month of treatment.

When samples were similarly tested in parallel with Thrombotest and Geigykinase Thrombotest consistently showed the treatment to be more effective than did Geigykinase. Tests with micro methods employing these two preparations gave results that were in fairly good agreement with those obtained with the standard methods.

The venous blood of 11 patients was systematically tested during and after short term phenylindanedione treatment. The above-mentioned findings regarding

Discussion

Until recently Idokinas has probably been the most commonly used thromboplastin preparation in Finland The present study revealed that compared with Geigykinase its use leads to an under dosage in the first days of phenylindanedione treatment to a similar dosage during the rest of the first month and later to a dosage so high that there is a danger of bleeding Compared with Simplastin it always leads to underdosage and compared with Thrombotest to high dosages.

Geigykinase which is lung thromboplastin and not brain thromboplastin is less sensitive to factor VII and more sensitive to factor X than the other thromboplastins examined (36 49) During the first days of phenylindanedione treatment it leads to the same conclusions as Simplastin later during the first month the results are the same on an average as those obtained with Idokinas. Subsequently it leads to a lower dosage than do Simplastin and Idokinas. Compared with Idokinas it leads to a danger of bleeding on the first days of treatment, but compared with Thrombotest it always does so The micro method with Geigykinase gave results that are closely comparable with those obtained with the standard method was easy to learn and saved much time.

Thrombotest is not actually a thromboplastin preparation alone for it contains in addition to brain thromboplastin and calcium also cephalin and bovine adsorbed plasma It is the newest of the thromboplastin preparations examined (27 28) As it can be used in a very handy micro method it has lately received much attention (2, 4 18 37 51) It has been claimed to be sensitive even to factor IX, which has not been claimed for any other thromboplastin preparation because thromboplastin itself is considered to possess factor IX activity Nothing can be learned about this question from the present study Owren (27) stated that the factor IX content fell already on the second day of anticoagulant treatment and was very low on the eighth to tenth days when the treatment was ended (as in the case of factor X in the present study) but did not mention factor X at all. Sise et al. (39) noted that the factor IX content began to decrease only in the second week. Opinions on the theoretical, practical and economic value of Thrombotest still vary greatly (1 14 15 16, 19 28 29 30 34 41 42 46)

Compared with the other thromboplastin preparations examined Thrombotest led to a quite distinct underdosage this is in close agreement with the results of Allington (2) Lempert and Poller (16) Moore and Beeler (23) Rodman and Pastor (35) and Tat and Lewis (43) The micro method employing Thrombotest is from the point of view of simplicity and accuracy about as good as the micro method employing Geigykinase

Simplastin like Geigykinase and Thrombotest, contains an adequate amount of calcium in addition to the thromboplastin. On the other hand no micro method based on Simplastin is available. Moreover Simplastin guides to a higher dosage than any of the other thromboplastins tested

In summary it may be said that the thromboplastin preparations examined give a rather different picture of the intensity of the anticoagulant treatment. This phenomenon, which has been reported previously (8, 49) may at least in part be the reason for the widely varying percentages of bleeding and variable

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the relationships between Idokinas Geigy kinase and Thrombotest were confirmed and moreover it was found that Simplastin led to a higher dosage than any of the other thromboplastins tested Compared with the other thromboplastins Geigy kinase yielded Quick values that were higher in the first week than in the second week and higher in the first month than later during the treatment.

The HRCT was found to be fairly well correlated with the Quick values but the initial HRCT's varied greatly

The study of the coagulation factors revealed that the level of factor VII decreased first and then the levels of prothrombin and factor X the level of the last mentioned factor was lower in the second week than in the first week of treatment whereas the levels of prothrombin and factor VII were at a minimum during the first week.

The Quick values obtained with the different thromboplastin preparations, the Thrombotest values and the levels of different coagulation factors rose after the treatment ended in nearly the same order as they had declined at the beginning of the treatment

Acknowledgment

The author wishes to express his deep gratitude to the Central Laboratory of Finland' Red Cross Blood Service for donation of cephalin and substrate free from factor VII This investigation was aided by a grant from Yrjo Jahnsson Foundation.

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From the Central Laboratory Kommunehospitalet Århus, Denmark

Studies on the Osmotic Fragility of Normal Human Erythrocytes

I. A Method for the Determination of the Effect of Hypotonic Solutions

By

Esper Mortensen

During the last few years the demonstration of shortened red-cell life span and enhanced haemolysis in various types of anaemias has blurred the boundaries between the classic types of haemolytic anaemias and various other diseases. Most of the methods of investigation applied in the determination of red-cell life span necessitate the use of radioactive isotopes and specialized equipment, and are based upon epeated rather delicate and time-consuming measurements. Consequently the application of these procedures is restricted to research work and special cases, leaving the clinician without this help for routine work.

In many cases only moderate degrees of shortening of the ed-cell life span will be found. Consequently the determination of serum biliruban, faecal urobilinogen excretion, and serum haptoglobin will not be sufficiently sensitive. The value of the reticulocyt count is reduced by the fallacies of the method (8) and by the dependence of the reticulocyte count on the erythropoiesis and not upon the destruction of the erythrocytes.

Theoretically the osmoti fragility test will effect changes in red-cell life span. However the use of the test for more than 50 years has yielded only meagre results. Significant deviations from the normal values are limited to the well known types of haemolytic anaemia. Divergent results in various states of disease probably reflect the variability and crudeness of the techniques applied. Normal results, in cases where enhanced haemolysis is evident, may reflect the uncertainty of the methods too The great variability of normal values and the divergence between the normals of various investigators stresses the importance of making the technique of the osmotic fragility test more exact.

The following investigations were carried out in order to elaborate an improved osmotic fragility test and to obtain reliable normal values.

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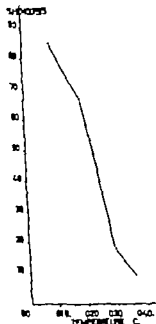


Fig. 2. The influence of temperature on the course of haemolysis in solution of 0.137 osmol. at pH of 7.40. The values are the means of several experiments to be reported later

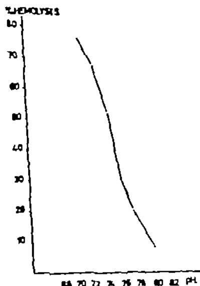


Fig. 3. The influence of the pH of the solutions on the course of haemolysis in solution of 0.137 osmol. at temperature of 10° C. The values are the means of several experiments to be reported later

thus evident. The simplest way of obtaining this is to buffer the haemolytic solutions. Unfortunately the osmolarity allows of only poor buffering capacity if NaCl is an indispensable component of the solutions. The replacement of NaCl by NaH_2PO_4/Na_2HPO_4 does not introduce new cations, but of the anions the chloride ion is reported (11) to pass the erythrocyte membrane somewhat faster than the phosphate ions. This may introduce a new variable factor in the haemolytic system.

If the ratio blood/haemolytic solution is high the large buffering capacity of the blood will easily overwhelm that of the haemolytic solutions. The resulting shift of the pH will vary unless the blood is standardized (see below). It is advisable, therefore, to buffer the solutions according to the degree of haemolysis expected and to keep the amount of blood added as small as possible. Apart from the CO_2/HCO_3 system the haemoglobin makes the main contribution to the buffering capacity of whole blood. The base-binding capacity of haemoglobin varies with the degree of oxygenation and the amount of carbhaemoglobin (9). If the CO_2/HCO_3 is removed and the haemoglobin saturated with oxygen, the buffering capacity of the blood is almost constant and there will be minimal variability in the actual pH of the haemolytic system. The standardization of the blood is accomplished by rotating the blood in smooth cylindrical glass tubes (10 × 3 cm) at 60 r.p.m. for 10—15 min. in the horizontal position. The layer of blood will be thin and its surface large. The haemoglobin will then show 100 % saturation with oxygen, the pH will have changed from about 7.3 to a value > 7.85—90 corresponding to equilibration of the CO_2/HCO_3 system with the CO_2-tension of room-air. Fig. 4 shows the difference between "mixed venous blood" and "standardized blood". In the present investigation the solutions applied are buffered with NaH_2PO_4/Na_2HPO_4 according to table II. A ratio

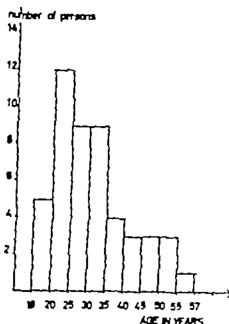


Fig 1 The age distribution of the normal persons examined.

Table I The laboratory tests applied and their normal range (95 %)

Determination of	Method applied	Normal range
Haemoglobin	Drabkin	The haematological values are identical with those of Wintrobe (15)
Erythrocyte count	Conventional	
Leukocyte count	Conventional	
Reticulocyte count	Conventional	
Haematocrit	Micromethod	
ESR	Westergren	2–10 mm/h
Serum bilirubin	Jendrassik & Grof	< 1.0 mg%
Serum creatinine	Bosnes & Taussky	< 1.5 mg%
Plasma iron	Sobel & Chiamori	52–198 μg%
Iron-binding capacity of plasma	Sobel & Chiamori	257–379 μg%

Material

Blood from volunteer blood donors was used throughout the investigations. Fourty nine men and women were examined. The age distribution is seen from fig 1 None of the persons investigated had been used as blood donors during the last six months before the trial or was suffering from acute illness on the day of investigation

The following analyses have been carried out as a routine concomitantly with the fragility test determination of haemoglobin, ESR, erythrocyte count, leukocyte and differential count, reticulocyte count haematocrit, serum bilirubin, serum creatinine, plasma iron and iron-binding capacity

All the values obtained were within the normal limits of the methods applied, and no person (table I) was excluded from the test group

Methods

The fragility test which has been elaborated aims to take account of all the important variables influencing the process of osmotic haemolysis

Temperature

The basic principle is that increasing temperature reduces the degree of haemolysis, whereas a lowering will result in enhanced haemolysis (5, 6 10) This is evident from fig 2 Under the stated circumstances a change of temperature from 15 to 25 C. will reduce the degree of haemolysis about 55 %. An important source of error is no or insufficient preheating of the solutions to the selected temperature. Blood must not be added till their temperature has been checked. The solutions are usually stored in the refrigerator between experiments and serious errors may consequently arise from negligence at this point of the procedure.

The reported investigations were carried out at a temperature of 37° C.

pH

The basic principle here is that a rise in the pH of the solutions will bring about a reduction of the degree of haemolysis, whereas a fall of the pH will increase it (6, 10) From fig 3 it may be seen that under the conditions adopted a shift of the pH from 7.2 to 7.6 will cause a reduction of the degree of haemolysis of about 40 %. The need of pH constancy is

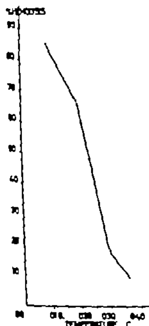


Fig. 2. The influence of temperature on the course of haemolysis in solution of 0.137 osmol. at pH of 7.40. The values are the means of several experiments to be reported later.

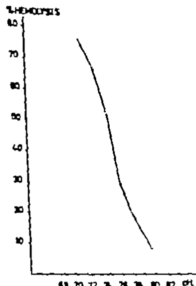


Fig. 3. The influence of the pH of the solutions on the course of haemolysis in solution of 0.137 osmol. at temperature of 10° C. The values are the means of several experiments to be reported later.

thus evident. The simplest way of obtaining this is to buffer the haemolytic solutions. Unfortunately the osmolarity allows of only poor buffering capacity if NaCl is an indispensable component of the solutions. The replacement of NaCl by NaH_2PO_4/Na_2HPO_4 does not introduce new cations, but of the anions the chloride ion is reported (11) to pass the erythrocyte membrane somewhat faster than the phosphate ions. This may introduce new variable factor in the haemolytic system.

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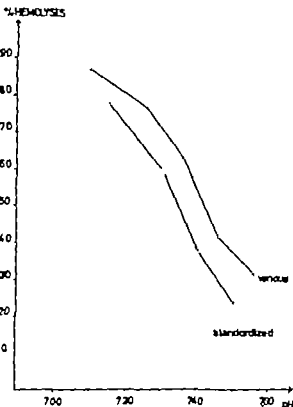


Fig 4 The influence of standardization of the venous blood to 100 % saturation of oxygen and the pCO of room air

blood/solution of 100/5 000 was chosen as appropriate Heparinized blood obtained by venipuncture was used, as it has been shown to resemble unstabilized blood in fragility qualities (2)

Table III shows the results of measuring the pH of venous blood, standardized blood the solutions applied, and the mixtures of blood and haemolytic solutions after 60 min. at 37 C. All the values are between pH 7 40 and 7 48.

The time

The process of haemolysis must be continued at least to the point of equilibrium, which depends upon the temperature and osmolarity of the solutions (6 10) During the investigations here reported the time of haemolysis was extended to 60 min. Control experiments have shown that full equilibrium has been attained at this time in all the solutions at 37° C.

The age of the blood

The fragility of the erythrocytes increases with the in-vitro-age and fragility tests must be carried out as soon as possible after the venipuncture. All the experiments reported below were performed within two hours after the venipuncture. The importance of this point is illustrated by fig 5 which shows the course of haemolysis of the same blood sample when the test was performed at once and after 24 hours at 37 C. Further experiments into this important point are being carried on, and will be reported later

The amount of blood added

will influence the haemolytic process and must be kept constant. In the results reported below the osmolarity has not been corrected for the change induced by the added blood but corrections can easily be calculated assuming the osmolarity of normal blood to be equal to 0.85 NaCl-solution.

Anaemia

will always influence the fragility test. During standardization of the blood the erythrocytes in an anaemic sample will shrink more than in samples with normal haemoglobin concentrations (2) Anaemic blood will consequently appear more resistant to hypotonic solutions. If the amount of blood added is large the difference in concentration of plasma will affect the osmolarity of the haemolytic solutions. In the present experiments only normal blood was used and no corrections had to be applied.

The composition of the haemolytic solutions

appears from table II

The calculation was done by means of the common buffer equation $pH = pK + \log \frac{C_B}{C_A}$ where C_B and C_A are the concentrations of base and acid respectively The pK of the acid must be calculated for each solution, correcting for the change of ionic strength according to the equation of Debye—Hugel $pK' = pK + (z-1) \times \frac{\sqrt{i}}{1+\sqrt{i}}$ where pK stands for the pK at ionic strength o and pK' = the corrected pK z = the charge of the acid anion and i = the ionic strength of the

Table II The composition of the haemolytic solutions

I	II	III	IV	V	VI	VII	VIII	IX	X
Equivalent to % NaCl	Osmolarity	Na_2HPO_4 (mMol/l)	NaH_2PO_4 (mMol/l)	NaCl (Mol/l)	Ionic strength	Corrected (pK)	Na_2HPO_4 (g/l)	NaH_2PO_4 (g/l)	NaCl (g/l)
0.20	0.0684	5.0	1.4418	0.0233	0.042	6.86	0.8900	0.1990	1.4615
0.26	0.0885	5.0	1.3459	0.0356	0.052	6.83	0.8900	0.1857	2.0811
0.28	0.0958	5.0	1.3151	0.0391	0.056	6.82	0.8900	0.1813	2.2845
0.30	0.103	8.0	2.0095	0.0373	0.063	6.80	1.4240	0.2773	2.1798
0.32	0.109	8.0	1.9637	0.0403	0.067	6.79	1.4240	0.2710	2.3693
0.34	0.116	8.0	1.9189	0.0441	0.070	6.78	1.4240	0.2648	2.5765
0.36	0.123	8.0	1.8871	0.0481	0.074	6.775	1.4240	0.2618	2.8118
0.38	0.130	8.0	1.8753	0.0511	0.077	6.77	1.4240	0.2588	2.9682
0.40	0.137	10.0	2.2386	0.0513	0.084	6.7"	1.7800	0.3089	2.9961
0.42	0.144	10.0	2.2129	0.0548	0.087	6.745	1.7800	0.3054	3.2033
0.44	0.150	10.0	2.1877	0.0578	0.090	6.74	1.7800	0.3019	3.3791
0.46	0.157	10.0	2.1581	0.0614	0.094	6.73	1.7800	0.2951	3.5866
0.48	0.164	10.0	2.1137	0.0649	0.098	6.725	1.7800	0.2917	3.7926
0.50	0.171	10.0	2.0894	0.0695	0.102	6.72	1.7800	0.2883	4.0599
0.52	0.178	10.0	2.0416	0.0720	0.104	6.71	1.7800	0.2817	4.2060
0.54	0.185	10.0	2.0182	0.0755	0.107	6.71	1.7800	0.2785	4.4119
0.60	0.205	5.0	1.0208	0.0940	0.110	6.71	0.8900	0.1409	5.4931
0.65	0.222	5.0	0.9976	0.1025	0.118	6.70	0.8900	0.1377	5.9911
0.70	0.239	5.0	0.9750	0.1110	0.127	6.69	0.8900	0.1346	6.4897
0.75	0.257	5.0	0.9311	0.1201	0.136	6.67	0.8900	0.1285	7.0175
0.80	0.274	5.0	0.9098	0.1286	0.145	6.66	0.8900	0.1256	7.5167
0.85	0.291	5.0	0.8892	0.1371	0.153	6.65	0.8900	0.1227	8.0155
0.90	0.308	5.0	0.8690	0.1456	0.162	6.64	0.8900	0.1199	8.5103

solution. Fig. 6 shows the relation between the ionic strength and the *pK*. If no attention is paid to the change of the *pK*, a serious error is introduced, resulting in a continuous change of the pH of the solutions.

The preparation of the solutions

The reagents used are NaCl (Merck p.a.), $Na_2HPO_4 \cdot 2H_2O$ (Merck p.a.) and $NaH_2PO_4 \cdot H_2O$ (Baker p.).

The samples were weighed on an analytical balance and dissolved in redistilled water in volumetric flasks. The pH of the solutions was checked after preparation and once a week. The following values were obtained in 33 measurements: range 7.40—46. Mean 7.44. S.D. 1.52. M ± 2 S.D. 7.41—7.47. The pH-measurements were performed with a glass electrode using NBS certified buffer as reference at 37° C.

Table III Control measurements of the pH of blood solutions and haemolytic mixtures performed at 37° C. Heparinized venous blood pH 7.25 standardized blood pH 7.90

Haemolytic solutions		Haemolytic mixtures	
%	pH	%	pH
0.26	7.43	0.26	7.45
0.36	7.44	0.36	7.44
0.46	7.44	0.46	7.46
0.56	7.40	0.56	7.46

Practical procedure

All tests should be carried out in duplicate. Centrifuge tubes containing 5,000 μl of the solutions 0.10—0.26—0.28— 0.60 % and

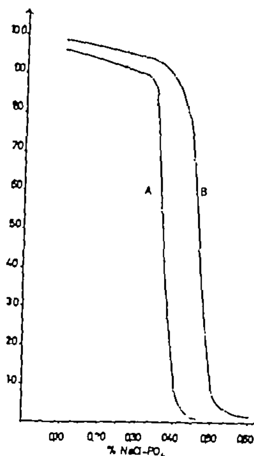


Fig 5 The influence of incubating the blood at 37° C for 24 hours. Curve A show the results of haemolysis performed immediately after the venipuncture. Curve B represents the course of haemolysis of the incubated blood.

0 % (redistilled water) are preheated to 37° C in a thermostat (maximal deviation < 0.50 C). Heparinized blood obtained at venipuncture is standardized as described above, and 100 μl is pipetted into each tube, which is stoppered and gently inverted 3—4 times to disperse the contents. The tubes are inverted every 15 minutes during the following 60 minutes to avoid sedimentation. The tubes are then centrifuged at 1 500 × G for 3 minutes and 1 000 μl of the supernatant fluid is pipetted into 5 000 μl Drabkin-solution. The optical density is determined with a photoelectric colorimeter at 524 nm.

The per cent haemolysis is calculated by dividing the optical density of the solutions by

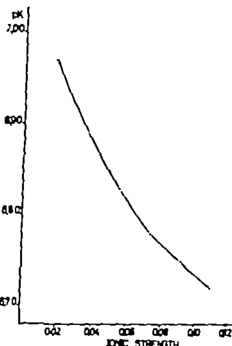


Fig 6. The relation between the pK of NaH_2PO and the ionic strength of the solutions applied. The figures were calculated from the Debye Hugel equation.

Table IV The variability of the osmotic fragility test described-expressed by the variability of duplicates. The values were calculated from 30 consecutive pairs of values of the optical density for the haemolytic ranges 50—100% and 0—50 %

	50—100 % haemolysis	0—50 % haemolysis
S. D.	1 40	1 15
Mean	298	44
S. D. × 1 4	4 08	2.1
Coefficient of variation	1.35 % (95)	4.7 % (95%)

the density of the 0 % solution and multiplying the resulting ratio by 100. The percentage of haemolysis in distilled water is hereby defined as being 100.

The precision of the method as expressed by the variability of duplicates is evident from table IV

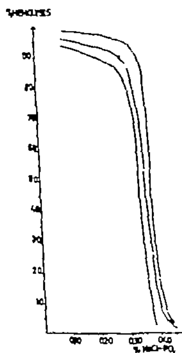


Fig. 7. The normal values of the osmotic fragility. The figures are based upon determinations on 49 normal persons. The mean and 95 % range is shown.

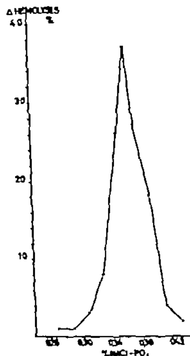


Fig. 8. The distribution curve of haemolysis. The increments of haemolysis were calculated from table V.

Results

The normal values obtained by the method described are recorded in figs. 7 and 8 and tables V and VI. The usual methods of recording the results have been followed. However as the initial haemolysis and 100 % haemolysis" levels are ill-defined, the author has recorded the 20 and 80 % haemolysis range together with the mean cellular fragility" (50 haemolysis range).

Discussion

The variables influencing the osmotic fragility of the erythocyte have been the object of intensive investigations for almost a century. Knowledge of the qualitative effect of these factors has been obtained, but a quantitative evaluation of the factors has been very difficult to achieve. Several investigators have succeeded in varying only a few factors keeping the other variables constant. Unfortunately the results thus obtained are valid only for the particular constants chosen, and would have been different if the value of only one of these constants had been otherwise fixed. The systematic evaluation of only the most important factors would require an enormous number of experiments. If the effect of the factors O —CO —pH—temperature and osmolarity were considered, an evaluation of each factor at ten levels, for instance would necessitate 10⁵ experiments. The

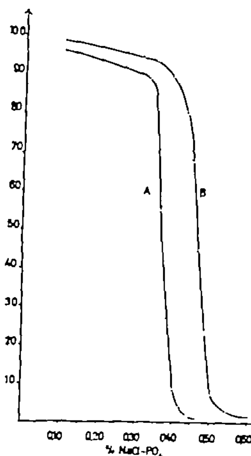


Fig 5 The influence of incubating the blood t 37° C for 24 hours. Curve A shows the results of haemolysis performed immediately after the venipuncture. Curve B represents the course of hae molysis of the incubated blood.

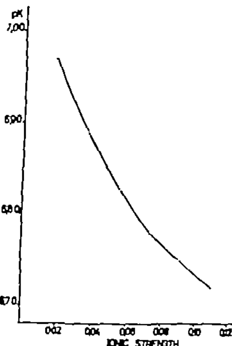


Fig 6. The relation between the pH of NaH_2PO and the ionic strength of the solutions applied. The figures were calculated from the Debye Hugel equation.

0 % (redistilled water) are preheated to 37° C in a thermostat (maximal deviation < 0.50 C) Heparinized blood obtained at venipuncture is standardized as described above and 100 μl is pipetted into each tube which is stoppered and gently inverted 3—4 times to disperse the contents. The tubes are inverted every 15 minutes during the following 60 minutes to avoid sedimentation. The tubes are then centrifuged at 1,500 × G for 3 minutes and 1 000 μl of the supernatant fluid is pipetted into 5,000 μl Drabkin-solution. The optical density is determined with a photoelectric colorimeter at 524 nm.

The per cent haemolysis is calculated by dividing the optical density of the solutions by

Table IV The variability of the osmotic fragility test described-expressed by the variability of duplicates. The values were calculated from 30 consecutive pairs of values of the optical density for the haemolytic ranges 50—100% and 0—50 %

	50—100 % haemolysis	0—50 % haemolysis
S. D	1 40	1 15
Mean	298	44
S. D. × 1 4	4.08	2.1
Coefficient of variation	1.35 % (95%)	4.7 % (95%)

th density of the 0 % solution and multiplying the resulting ratio by 100 The percentage of haemolysis in distilled water is hereby defined as being *100*

The precision of the method as expressed by the variability of duplicates is evident from table IV

of the pH as the osmolarity is varied, has not been corrected (3).

Levinson and MacFate in their book "Clinical laboratory methods" (7) suggest the method elaborated by Suess et al. (12) according to which the haemolytic solutions are allowed to vary between a pH of 5.5 and 7.0 and the temperature is "room-temperature" which may vary from 18—25° C in most European countries.

In 1936 Emerson et al. introduced a conversion-chart for pH-osmolarity changes (4). Unfortunately the technique of haemolysis applied displays several serious errors. The temperature is not stated. The ratio blood/haemolytic solutions is high, the blood unstandardized and the solutions unbuffered. The pH of the mixture of blood and solutions will accordingly show great variability. The authors have measured the pH of the blood just before transferring it to the solutions, but no constant relation between the pH of the blood and the pH of the haemolytic mixtures exists, and the values reported in the chart are not based upon the actual values. The time allowed for the haemolytic process to attain equilibrium is not fixed. The tubes are centrifuged and the haemoglobin concentration determined "soon after" the blood was added, whereby another variable factor is introduced.

The question of whether it is possible to construct a conversion chart on the basis of a linear relationship between erythrocyte-volume and changes of pH and osmolarity is difficult to settle. One of the authors (W. B. Castle) showed in 1937 (1) that a nonlinear relationship exists between the per cent change in erythrocyte volume and equal changes in pH or osmolarity. The final solution of this question will require more systematic investigations into MCV variations.

Various objections may be raised against the osmotic fragility test here elaborated. The ratio chloride/phosphate is varying in the solutions. However, the advantage of the pH-constancy thus achieved will probably outweigh this. The cyanmethaemoglobin-method necessitates a high ratio blood/haemolytic solution, but the frequency of methaemoglobinaemia, and the fact that the most fragile erythrocytes may hold more methaemoglobin than the resistant ones, demands a method of determination which includes this form of haemoglobin (13).

All investigators fail at one or several points in elaborating "the perfect osmotic fragility test". However, considering the amount of knowledge of the variable factors influencing the haemolytic process which has been available for more than 50 years, one cannot help wondering why this knowledge has not been applied. In 1931 Jacobs and Parpart had to admit (6): "It is disheartening to be forced to believe that a large part of the work in this much cultivated field is of very doubtful value, but there seems to be no escape from such a conclusion." This statement apparently still holds true.

Summary

The osmotic fragility test will be greatly influenced by the temperature and pH of the solutions, by the amount of haemoglobin added, the pCO_2 and the degree of oxygen saturation of the haemoglobin, by the time allowed for the haemolysis, and by the in vitro age of the blood. An osmotic fragility test that takes these factors into account has been elaborated, and normal values based upon determinations on blood from 49 healthy

Table V Osmotic fragility normal values based upon determinations on blood from 49 normal men and women in the age range 20—55 years

Equivalent to % NaCl	Mean	S. D.	% haemolysis 95 % range
0.10	96	1 4	98.8—93.2
0.26	92	2.2	96.4—87 6
0.28	91	2 1	95.2—86.8
0.30	90	2.7	95.4—84.6
0.32	87	3.9	94.8—79.2
0.34	79	8.9	96.8—61.2
0.36	53	17.5	88.0—18.0
0.38	26	12.0	50.0— 2.0
0.40	8	4.7	17 4— 0
0.42	4	1.5	7.0— 1 0
0 44	2	0.8	3.6— 0.4

Table VI Osmotic fragility normal values (95 % range) The figures were extracted from fig 7

	Osmolarity expressed as % NaCl-solution
80 % haemolysis	0.325—0.375
50 % haemolysis	0.345—0.380
20 % haemolysis	0.360—0.395

amount of blood required could not be obtained from one person and the effect of the in vitro age of the blood would greatly influence the results.

The recognition of pro-haemolytic and anti haemolytic factors together with a set of currently approved standard conditions must be the basis for an acceptable test of osmotic fragility in which only osmolarity is varied the other factors being kept constant Unfortunately in the most commonly employed methods other factors are varied—sometimes erratically and unknowingly

In 1938 the photoelectric determination of the degree of haemolysis was introduced by Waugh and Asherman (14) and since then methods of this type have prevailed.

In 1947 Parpart et al published their well known osmotic fragility test which is still commonly used. The method (10) acknowledged the important factors and tried to vary only osmolarity The haemolytic solutions were buffered with NaH_2PO_4/Na_2HPO_4 and calculated to have a pH of 7 4 Unfortunately an important error was introduced in elaborating the technique in preparing the solutions from a 10 % stock-solution" by dilution the pH is changing as the ionic strength and the pK are reduced (cf. fig 6) The error is theoretically about 0 4—0.5 of a pH unit. When measuring the pH an even greater error will be found As stated by Parpart et al. (p 639) a change of pH by 0 1 of a pH unit is equivalent to altering the salt concentration by 0 01 % The more dilute the solutions, the higher the pH and the degree of haemolysis obtained will be smaller than at constant pH values. Another important source of error is introduced by the dilution technique the buffering capacity is steadily reduced as haemolysis increases with the degree of hypotonicity A great variability of the pH in the mixtures of blood and haemolytic solutions will result, especially as no attempt was made to standardize the blood. No actual pH values were measured

Wintrobe in Clinical haematology" (15) suggests a modification of the method of Parpart et al. The method offers no advantage the errors being unaltered

Dacie in his book "The hemolytic anemias recommends another modification of the Parpart technique The fundamental error uncontrolled variability

Acta Medica Scandinavica. Vol. 173, fasc. 6, 1963

From the Central Laboratory Kommunehospitalet, Århus, Denmark

Studies on the Osmotic Fragility of Normal Human Erythrocytes

II. A Method for the Determination of the Effect of Temperature on the Fragility of Erythrocytes

By

Esper Mortensen

Osmotic fragility tests have been in common use in the clinical laboratory for almost half a century. Numerous modifications of the test are—or have been—in common use, resulting in great difficulties in interpretation and comparison of the results of various investigators. Two different sorts of modifications exist. Some authors try to simplify the test in order that it may be more commonly used, while others refine the test to obtain less variability with the method. The performance of a correct osmotic fragility test is rather complicated since it is essential to take account of the variable factors influencing the course of hemolysis. The demand of pH constancy of the hemolytic solutions results in the necessity of preparing each solution separately and checking the pH at regular intervals while storing.

During evaluation of the influence of temperature on the course of hemolysis, a method of determining the temperature fragility of normal human erythrocytes was elaborated. With this method only one hemolytic solution is applied whereby an important simplification of the practical procedure is achieved without loss of precision. The details of the method and the values obtained in 20 normal persons will be reported. For a detailed discussion of the variable factors influencing the hemolytic process the reader must be referred to a previous paper (3).

Material

Blood from healthy volunteers was used throughout the investigation. 20 persons in the age range 18—55 years were examined. The following laboratory tests were performed on all samples of blood: determination of haemoglobin, erythrocyte count, leukocyte count, reticulocyte count, haematocrit, ESR, serum bilirubin, serum creatinine, plasma iron and total iron-binding capacity of plasma. All the values obtained were within the normal limits of the methods applied (table I).

Submitted for publication December 3, 1962.

men and women in the age range 20—55 years are given.

The methods most commonly applied are critically reviewed

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Table I The laboratory tests applied and their normal range (95 %)

Determination of	Method applied	Normal range
Haemoglobin	Drabkin	The hae-
Erythrocyte count	Conventional	matological values are
Leukocyte count	Conventional	identical
Reticulocyte	Conventional	with those
Haematocrit	Micromethod	of Wintrobe
ESR	Westergren	*2–10 mm/h*
Serum bilirubin	Jendrassik & Grof	<1 0 mg%
Serum creatinine	Bonnes & Trousky	*<1.3 mg%*
Plasma iron	Sobel & Chlamori	52–198 $\mu g\%$
Iron-binding capacity of plasma	Sobel & Chiamori	257–379 $\mu g\%$

Table II The composition of the haemolytic solution

Concentration	Na_2HPO_4	NaH_2PO_4	NaCl
mMol/l	10.00	2.2386	51.30
g/l	1 7800	0.3089	2.9961

Ionic strength = 0 084
pK = *6.75*
Osmolarity = 0 137 equivalent to 0.40% NaCl.
pH of the solution = 7 40 – 7 44

Methods

All samples were subjected to an osmotic fragility test which has previously been described (3) and to a thermoresistance test.

The principle of the test is as follows the factors influencing the in vitro haemolytic process — viz. osmolarity pH of the haemolytic mixtures, the ratio blood/haemolytic solutions, the degree of oxygenation of the haemoglobin the pCO_2 of the haemolytic mixtures, the time for haemolysis, and the in vitro age of the blood — are fixed except for the temperature, which thus becomes the independent variable of the system. The dependent variable — viz. the degree of haemolysis obtained — is measured by centrifuging the tubes with the haemolytic mixtures, and the haemoglobin concentration of the clear supernatant fluid is then determined as cyanmethaemoglobin.

The osmolarity of the solutions was fixed at 0 137 osmol. which is equivalent to a 0.40 % NaCl-solution. No correction for the change of osmolarity induced by adding the small amount of blood (100 μl) was introduced, as this change will be constant for all practical purposes, when working with normal blood. If the osmolarity of normal blood is stipulated as being equivalent to a 0 85 NaCl-solution, the correction can easily be calculated.

The pH of the solutions. the solution is buffered with NaH_2PO_4/Na_2HPO_4 to a pH of 7 40 at 25 C. The buffering capacity of the solution is small as NaCl was intended to be preserved as the principal component of the osmolarity A concentration of Na_2HPO_4 of 10 mMol/l was arbitrarily fixed and the concentration of NaH_2PO_4 was calculated by means of the common buffer equation. The pK value applied was corrected according to the Debye—Hügel equation to agree with the ionic strength of the solution. The composition of the solution and its pH which was checked after preparation and regularly during use, are given in table II.

It must be acknowledged that the shift of temperature between 0 and 40° C will influence the pH (the error will be small and constant from experiment to experiment) the temperature-coefficient being about 0 002 of a pH unit/° C. The values actually differed less, probably as a result of the influence of the buffering capacity of the haemoglobin.

The blood added was heparinized blood obtained at venipuncture. On account of the effect of the in vitro-age of the blood (3) the experiments were performed during the first few hours after the venipuncture. 100 μl blood was added to each of the tubes.

The time was chosen as that shown experimentally to be needed for the attainment of equilibrium in the haemolytic system. The lower the temperature, the longer the time (4) For temperatures above 10° C 60 min. were fixed, and for 10° C and below 150 min. were used.

Oxygen saturation and CO_2-tension of the blood added must be fixed on account of the great buffering capacity of the blood itself, which

will influence the final pH of the haemolytic mixture and cause a shift of the pH. This shift will depend upon the degree of oxygen saturation and the pCO_2 of the blood, as these values determine the actual base-binding capacity of the haemoglobin. By rotation in open vessels (3) the blood can be standardized to an oxygen saturation of 100 % and pCO_2 equal to that of room air. Hereby a constant and rather small change of the pH will be achieved and the final pH of the haemolytic mixtures will vary insignificantly from experiment to experiment.

The ratio blood/haemolytic solution must be kept constant from experiment to experiment — partly because of the change of the pH which will increase with the amount of blood, and partly because of the need for keeping constant the effect of the blood on the final osmolarity.

Anaemia and polycythemia will thus influence the degree of haemolysis obtained (2). The simplest way of correcting this effect will be the adjustment of the haematocrit to normal values by removing or adding plasma. In the present test 100 µl of blood was added to 5,000 µl of the haemolytic solution.

The preparation of the haemolytic solution

The reagents were weighed on an analytic balance and dissolved in redistilled water in volumetric flasks. The salts used are NaCl (Merck p.a.), Na_2HPO_4, $2H_2O$ (Merck p.a.) and NaH_2PO_4, H_2O (Baker p.a.)

The pH control of the solutions was performed with glass electrode using an NBS certified buffer as standard reference solution.

The temperature

In this test the variation of temperature produces the variation in the degree of haemolysis. Seven levels of temperature were applied viz. 0—5—10—20—25—37—40° C resulting in percentages of haemolysis between 0 and 90. Sufficient variations in the dependent and independent variables are thus achieved to establish the relation between temperature and haemolysis. If greater osmolarity or higher pH had been applied the percentages of haemolysis would have been smaller. If lower osmolarity or lower pH had been used higher percentages of haemolysis would have been obtained.

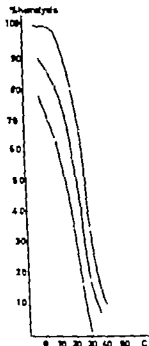


Fig. 1. The normal values of the thermo-resistance test. The figures are based upon determinations on blood samples from 20 healthy men and women aged 20—55 years.

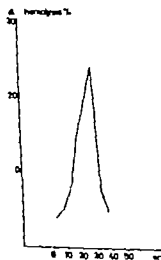


Fig. 2. The distribution curve of haemolysis with the thermo-resistance test. The values were extracted from fig. 1.

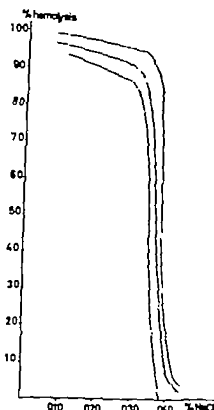


Fig 3 The normal values of the osmotic fragility test applied. The values are based upon determinations on blood samples from 49 healthy men and women aged 20—55 years.

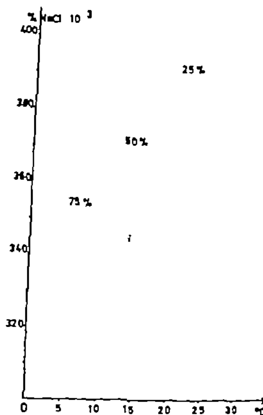


Fig 4 Scatter-diagram correlating the osmotic and thermal fragility of normal blood at the levels of 25—50—75 haemolysis.

The temperature limits of 0 and 40° C are fixed by the destruction of the red cells which will occur at higher or lower temperatures (5)

The various temperature levels may easily be obtained even in the routine laboratory 0° with a mixture of ice and water 5 in the refrigerator 10° in streaming/running tap water 20° as room temperature, 25—37 and 40° by means of thermostats. It is most important that the solution should be adjusted to the appointed level and that the actual temperature of the haemolytic mixtures during the test be measured since significant variations of the degree of haemolysis may be obtained even with small variations of temperature. The results of the test are plotted in a rectilinear coordinate system with the temperatures as abscissae and the haemolysis as ordinates. The actual temperatures should of course be plotted if diverging from those stipulated.

Practical procedure: all tests were carried out in duplicate. Centrifuge tubes each containing 5 00 ml of the haemolytic solution are adjusted to the temperature levels chosen viz. 0—5—10—20—25—37 C. Heparinized venous blood is standardized according to the method previously reported (3) and 100 μl blood is added to each tube and to tubes containing 5 00 ml distilled water The tubes are stoppered and gently shaken by inversion immediately and every 15 min. during the test. When the period of haemolysis has elapsed the tubes are centrifuged at 1 500 G for 3 min. and the haemoglobin concentration of the supernatant fluid is determined by pipetting 1 000 μl into 5,000 μl of Drabkin s solution. The optical density is determined with a photoelectric colorimeter at 524 mμ.

The percentages of haemolysis were calculated by dividing the optical density of each tube by the optical density of the tube with distilled water and multiplying the ratio by 100. The percentage of haemolysis in distilled water is hereby defined as being 100 %.

Table III. The normal values of the thermo-resistance test. The figures were calculated on the basis of determinations on blood from 20 healthy persons

t (°C)	Mean	S. D.	95 % range
0	90	6	100—78
5	86	8	100—70
10	81	8.5	98—64
20	58	11	80—36
25	42	12.5	67—17
30	18	8.5	35—1
37	7	4	15—0
40	5	2.5	10—0

Results

The normal values of the thermo-resistance test are recorded in fig. 1 and table III.

The increments of haemolysis with alteration of the temperature of the haemolytic mixtures may be calculated from fig. 1 and the results plotted in a coordinate system with the % increments as ordinates and the temperatures as abscissae (fig. 2). This curve will represent the change of haemolysis with temperature.

The results of the concomitantly performed osmotic fragility test all fell within the normal limits of the method (fig. 3). To show the correlation between the two tests a scatter-diagram was constructed (fig. 4) and the coefficient of correlation was calculated for the 50 % haemolysis level and found to be 0.94. It is evident that for normal blood a high degree of correlation exists between the two tests. The variability of the method appears from table IV.

Discussion

Although this test is easy to perform the variable factors influencing the course of haemolysis have to be kept constant.

Table IV. The variability of the method expressed by the variability of duplicates. The values (95% range) were calculated from 25 consecutive pairs of values of the optical density for the ranges 50—100% and 0—50% of haemolysis

	50—100	0—50
S. D	1.64	1.15
Mean	204	39
S. D × 1.4	2.30	1.61
Coefficient of variation	1.13 %	4.13 %

The correlation between the tests may indicate that the thermo-resistance test can be used instead of the osmotic fragility test, which is more laborious to perform. The degree of correlation between the two tests in states of disease, however, is not known and must be determined before a final evaluation may be safely done.

Summary

On the basis of experiments a test of the thermal fragility of red blood cells is elaborated and normal values determined in 20 healthy persons are recorded.

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Malabsorption Induced by Small Doses of Neomycin Sulphate

By

Steffen Hvidt and Knud Kjeldsen

Neomycin sulphate is at present widely used for the preoperative sterilisation of the alimentary tract and in the treatment of hepatic coma.

With peroral administration, only a small quantity of the neomycin is absorbed (8) hence this rather toxic substance has only in a few cases given side effects other than diarrhoea.

Used even for short periods, neomycin often gives abnormal growth of fungus in the intestines, but only a few cases of staphylococcal enterocolitis have been published (17).

Faloon et al. (7) have shown that neomycin sulphate, in doses of 12 g daily perorally could produce a malabsorption syndrome assessed by the carotene absorption, d-xylose absorption, fat absorption, B_{12} absorption 59 Fe-tests, glucose absorption and serum cholesterol-examinations. On a small number of patients, treated with 4—6 g neomycin sulphate daily Jacobson and Faloon (6, 12) found a corresponding although less pronounced effect.

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The purpose of this paper has been to ascertain whether or not a dose of neomycin sulphate of 1 g t.i.d. per os, as routinely used in the treatment of hepatic coma in our department, could produce malabsorption.

Methods

The material consists of 10 men between the ages of 51 and 82 years admitted for coronary occlusion, without notable incompensation and without gastrointestinal or urological complications. The study was started as soon as the cardiac situation was stabilized.

The patients were on diet of 70 g fat per day during the study. Only occasionally a 20 % solution of magnesium sulphate 15 ml daily was used to regulate the bowels.

The examination was divided into three consecutive periods each of 10 days.

A. A primary control period.

B. A neomycin period — in which was administered 1 g neomycin sulphat t.i.d. (equivalent to 2.1 g neomycin base daily).

C. A final control period without neomycin.

Table II Urinary excretion of d xylose (g/24 hrs) before (A) during (B) and after (C) 5 g neomycin sulphate daily for 10 days Normal value 4.5—10.5 g/24 hrs

Patient No.	A	B	C
1	8.1–7.2 (7.65)	2.7–3.2 (2.95)	4.2– 5.6–6 3 (5.47)
2	7 4–5.7 (6.55)	1.8–2.7 (2.25)	3.4– 4.6–2 6 (3.5)
3	7.9–7.0 (7 45)	4 1–4 0–5.1 (4.40)	6.4– 2 0 (4.20)
4	10.0–6.2 (8.10)	1 1–1.0–2.4 (1.50)	4.6– 3.8–4.3 (4.23)
5	9.5–7.3 (8.40)	5.6–4.9–5.2 (5.23)	6.7–11.2–5 0 (7.63)
6	6.4–4 5 (5.55)	4.6–5.0–4.1 (4.57)	5.4– 6.6–7 6 (6.53)
7	6.8–6.4 (6.60)	3.3–4 4–4.3 (4.00)	5.6– 6.3–6 6 (6.17)
8	5 0–5.5 (5.25)	1.3–1.9–1.3 (1.50)	4.1– 4.0 (4.05)
9	7.8 5.8 (6 80)	6.0–4.7 (5.35)	6.6– 5.7–5.8 (6.04)
10	6.2–5.8 (6.00)	5.7–6.2 (6.05)	5.2– 5.0–5.2 (5.15)
Arithmetical mean	6.8	3.7	5.1
S. D.	± 1 4	± 1.6	± 1.8
S. E. of mean	± 0.31	± 0.32	± 0.33

Table III Serum cholesterol (mg/100 ml) before (A), during (B) and after (C) 5 g neomycin sulphate daily for 10 days Normal value 150– 300 mg/100 ml

Patient No.	A	B	C
1	201–223 (213)	201–210–226 (212)	212–233–300 (249)
2	358–323–333 (339)	303–282–284 (290)	262–305 (284)
3	226–201–212 (213)	174 170–173 (172)	181–179–210 (190)
4	269–249–226 (248)	230 206–200 (212)	220–250 (235)
5	308–300–282 (296)	246–240 (243)	225–245 237 (236)
6	168–188 202 (186)	197–174–183 (183)	186–196–222 (210)
7	197–190–206 (198)	172–172 (172)	182–198–185 (189)
8	309–294–312 (305)	293–255–248 (263)	248–244–266 (243)
9	325–297–297 (306)	288–240–241 (256)	255–281–265 (267)
10	463–424–408 (432)	345–350 (348)	340–296–344 (327)
Arithmetical mean	275	233	242
S. D.	± 76	± 53	± 46
S. E. of mean	± 14	± 10	± 8

8) have the increases been of such magnitude as to be interpretable as a sign of malabsorption.

There was a significant variation from the primary period to the neomycin period and from the neomycin period to the final period in the group as a whole (table V)

D-xylose absorption

Six patients (no. 1 2, 3 4 7 and 8) showed a considerable fall in the d xylose excretion to values under normal in the neomycin period, followed by an increase in the final period n all these patients with the exception of one (no. 3) The last xylose test from this patient was quite irregular and as the middle test

Table I Fat in stools (g/72 hrs) before (A) during (B) and after (C) 3 g neomycin sulphate daily for 10 days Normal value < 18 g/72 hrs

Patient No.	A	B	C
1	7 0	56.6	10.8
2	9 4	28.0	11 7
3	15.8	61 4	14.8
4	23.3	42.5	19 4
5	7.8	14 7	3.4
6	10.4	20.0	12.1
7	21 1	26.0	10.6
8	16.8	39.6	13.1
9	21.5	12.6	20 7
10	10 7	10.5	7 4
Arithmetical mean	14 4	31.2	12.4

Fat absorption

In the 5th to 8th day of each period the stools for a period of 72 hours, demarcated by the administration of carmine, were examined for total fat content. The analysis was carried out on wet stools using a modification of King's method (13) The upper limit of normal is considered, according to Hess Thaysen (16) to be 6 g per day the equivalent of a recovery percentage of approximately 91

D-xylose absorption

This was determined using a modification of the Roe and Rice method (5) on a 24 hours urine after the peroral administration of 25 g d xylose Normal values are 4.5—10 5 g (m ± 2 s.d)

The examination was carried out twice in the primary control period, on the 4th, 6th, and 10th day in the neomycin period, and on the 2nd, 5th, and 8th day in the final control period.

B_{12}-absorption

B_{12}-absorption was determined using Schilling's method (15) at the Medical Laboratory Research Department, Hellerup Middle value 20.5 % and lowest observed value 12 %

The test was carried out once in each period on the 6th, 9th, and 9th day respectively

Serum cholesterol

Serum cholesterol was determined using Carr and Drekter's method (2) Normal values 150—300 mg % (m ± 2 s.d.)

The tests were carried out on the 5th, 7th, and 10th day in the primary period, on the 4th, 7th, and 10th day in the neomycin period and on the 2nd, 5th, and 8th day in the final control period.

In each period bacteriological tests were carried out on the stools using blood and lactose-agar The number of stools and their appearance were noted daily

In the statistical evaluation the Students t-test has been used.

Results and discussion

The results are seen in tables I II III and IV

Fat absorption

Six patients (no 1 2, 3 4 6 and 8) showed a considerable increase in fat excretion in the neomycin period to levels above the upper normal limit In all these patients there was a fall in the final control period resulting in values on a level with those of the primary period.

One patient (no 5) excreted twice as much fat in the neomycin period without exceeding the normal value. The excretion fell below the original level in the final period

Two patients had unchanged values under neomycin treatment and one patient showed a fall

On practical grounds it was impossible in this study to collect stools for a period of more than 72 hours, even though a collection over for example, 5 days would give more reliable results. As in addition 3 patients had commencing values of between 7 and 8 g of fat in the stools per day without the cause of this being comprehended we have therefore considered that only in 5 patients (no 1 2 3 4 and

Table V. Results of statistical analysis with student t-test (f = 9)

	Period A B	Period B:C	Period A C
Fat in stools	t = 2.889 $0.02 > p > 0.01$ (s)	t = 3.410 $0.01 > p > 0.001$ ( )	t = 1.457 $p > 0.1$ (ns)
D-xylose excretion	t = 4.663 $0.01 > p > 0.001$ ( )	t = 4.530 $0.01 > p > 0.001$ ( )	t = 3.120 $0.02 > p > 0.01$ (s)
B_{12} ^{58}Co excretion	t = 2.105 $0.1 > p > 0.05$ (ns)	t = 0.547 $p > 0.1$ (ns)	t = 2.688 $0.05 > p > 0.02$ (ns)
Serum cholesterol	t = 4.860 $p < 0.001$ (s)	t = 1.452 $p > 0.1$ (ns)	t = 1.215 $p > 0.1$ (ns)

= significant, ns = not significant at the 0.02 level.

final control period. Seven patients showed a slight fall in the neomycin period, and 1 patient (no. 1) had an increase.

We have not thought that the fall in these 2 patients should be considered of any consequence, because there also in this diminutive material appeared one patient with an increase of similar magnitude, and because others (3) have found variations of up to 30 % of the initial value on repeated tests on the same patient.

On statistical evaluation this group as a whole showed no variation between the 3 periods (table V)

Comments

It has been considered whether or not the possible cause of the malabsorption demonstrated here could be a hypermotility of the intestine as diarrhoea is a frequent complication of neomycin treatment. We examined therefore the number of bowel movements in the primary and in the neomycin period and found that all the patients who showed signs of malabsorption had more passages in the neomycin period than in the primary period, and furthermore that these patients had more passages in the neomycin period than those who showed no signs of malabsorption.

Whether this hypermotility is the cause of malabsorption or hypermotility is caused by malabsorption or it is a concurrent phenomenon is impossible to say from our work. That malabsorption can occur without the presence of hyper motility can be seen by the fact that Jacobson et al. (10) found malabsorption in neomycin-treated patients suffering from obstipation.

Jacobson et al. (11) obtained biopsies from the intestine in 10 patients treated with neomycin and found in each patient changes in the mucosa of the small intestine similar to those found in idiopathic steatorrhoea. That idiopathic steatorrhoea and neomycin-induced malabsorption can hardly be the same condition is shown by the fact that treatment of the latter condition with a gluten-free diet did not reduce the fat excretion (9) This work points to the fact that neomycin has a toxic effect directly on the intestinal mucosa.

As the neomycin dosage used in the present work could hardly be expected to sterilize the intestinal tract (4) bacteriological investigations were done on the

Table IV 24 hrs urinary excretion of B_{12} ^{58}Co (%) before (A) during (B) and after (C) 3 g neomycin sulphate daily for 10 days Normal value > 12 %

Patient No.	A	B	C
1	6.4	13.9	5.5
2	9.3	9.1	11.0
3	25.1	12.9	24 4
4	22.4	21 4	15.8
5	23.8	15.9	15.9
6	25.2	22.4	23.5
7	29 6	12.1	18.5
8	18.7	16.4	14.8
9	23.7	19.5	27.0
10	24.2	21 4	19.1
Arithmetical mean	20.9	16.5	17.6

had not been taken owing to a mistake, it was impossible to evaluate the results from this patient in the final period

One patient (no 5) had a considerable fall in the neomycin period but without abnormal values being obtained the fall was followed by an increase in the final period

One patient (no 9) had a slight fall and 2 patients (no 6 and 10) had small increases in the neomycin period

Six of 10 of the examined patients thus showed definite signs of malabsorption with this test.

The variation between the primary and the neomycin period as also between the neomycin and final period was significant for the group as a whole. Also from the primary to the final period there was a significant variation thus pointing to the fact that the effects of the neomycin were still apparent in the final period (table V)

Serum cholesterol

Apart from 2 patients (no 1 and 6) who had unchanged serum cholesterol values, the whole group had a fall in the neomycin period However it can be seen from table III that the spontaneous variations in the primary period were considerable for the individual patient. As serum cholesterol in addition has a tendency to a spontaneous decline during hospitalization it was not considered that the fall for the individual patient in the neomycin period was large enough to give any definite conclusion In all probability our work has been of too short a duration to judge the full effect of neomycin on serum cholesterol, inasmuch as the maximum effect according to Samuel (14) appears after 1—3 weeks treatment with 1.5 to 2 g neomycin sulphate daily per os.

Of the 8 patients with a fall in serum cholesterol the one half had a slight decrease and the other half a slight increase in the final period As Samuel (14) however has shown that the neomycin effect on the serum cholesterol level is retained from 1 to 8 weeks after the administration of the drug has ceased our results in the final period can hardly be considered of value when judging the possible effects of neomycin.

That neomycin probably has had some lowering effect on the serum cholesterol level can be seen from the fact that the values in the primary control period and in the neomycin period had a significant difference at the $p < 0.001$ level (table V)

B_{12}-absorption

In the primary period there is found considerable variation in this test from patient to patient (6 4 % to 29 6 %) which is in accordance with normal laboratory experience (1) table IV

Only 2 of our patients (no. 3 and 7) showed a considerable fall which in both cases was followed by an increase in the

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feces in the neomycin period and showed a flora dominated by fungi and gram negative organisms in both patients with and patients without malabsorption this indicates that the intestinal flora has little or no effect with regard to the occurrence of malabsorption

The primary object of our work was to ascertain whether or not 3 g of neomycin sulphate was sufficient to produce malabsorption and as this appears to be the case the question then arises whether or not large and small doses of neomycin cause similar degrees of malabsorption. In order to throw light upon this, we have compared our material with that of Jacobson (10) who gave 12 g of neomycin sulphate daily We do not believe that the B_{12}-resorption and the serum cholesterol examinations done in both groups can be compared

The d xylose test provides the best comparative material even though Jacobson uses a 5-hour and we a 24-hour urine sample. Three of Jacobson s 8 patients showed abnormally low resorption against 6 of our 10 Stated in a similar manner to Jacobson the number of patients, who in the neomycin period had a fall of more than 35 % of the initial values in the d xylose test, there were 6 of 8 patients in Jacobson s material and 7 of 10 in ours. No difference in d xylose absorption cannot be seen between the two materials treated with 3 and 12 g neomycin sulphate daily

Whether the malabsorption demonstrated here caused by neomycin, has any real significance for the organism in the short periods that the drug is normally administered is doubtful. Until this has been closely examined one should probably only use neomycin in short periods and on occasions where it offers more advantages than other antibiotics.

Summary and conclusion

Ten patients without gastro-intestinal or urological diseases received for 10 days neomycin sulphate 1 g t.i.d. per os and were, during this period as well as in a primary and a final control period examined in order to see if a malabsorption syndrome developed the criteria used being fat-absorption d xylose-absorption, B_{12}-absorption, and serum cholesterol levels.

Five patients showed definite signs of malabsorption of both fat and d xylose whilst 1 patient showed a reduced d xylose absorption with a normal fat absorption. The remaining 4 patients retained normal absorption of both d xylose and fat. Serum cholesterol levels and B_{12} 58_{Co} absorption allowed no definite conclusions to be drawn with regard to the individual patient although the serum cholesterol showed a significant fall in the group as a whole during neomycin treatment.

The patients who showed signs of malabsorption passed a greater number of stools whilst under treatment with neomycin than the remaining patients.

A daily administration of 3 g neomycin sulphate per os is thus within 10 days able to produce malabsorption — an effect similar to that demonstrated by others with the administration of 12 g of neomycin sulphate

In the clinical use of neomycin this side effect should be remembered and the drug used only in periods as short as possible and only in situations where it offers more advantages than other antibiotics. It should also be remembered that it can be misleading to examine patients for malabsorption caused by other factors when neomycin is being administered

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Antibacterial Activity of Long-acting Sulfonamides

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With the advent of antibiotics the sulfonamides were pushed into the background. It did at times look as if there was no longer any place for them in clinical therapy.

Lately, however, the interest in sulfonamides has increased. This may be due firstly to the fact that it has gradually become clear that antibiotics also have their limitations (side effects, resistance) and secondly, that in recent years sulfonamides have been developed which have characteristics quite different from the earlier compounds.

Sulfadiazine has shown antibacterial properties which are hardly surpassed by any other sulfonamide. For this reason, sulfadiazine has kept its place as the sulfonamide to which the newer compounds are compared. The duration of the bacteriostatic effect of sulfadiazine is, however, short, and the newer long acting compounds are partly developed in order to make administration easier.

These long acting sulfonamides may be given with safety during long periods of time (7) and also in cases of moderate renal insufficiency as one of us has shown (8).

To the most thoroughly investigated of the long-acting sulfonamides belong sulfamethoxypyridazine, sulfadimethoxine and sulfaphenazol. They are all bound to the serum proteins to a greater extent than is sulfadiazine.

Since the 1940's one has assumed that the protein-bound fraction of a sulfonamide has no antibacterial effect (3) and this has also been put forward in recent publications (2, 9). In contrast to this, it seems as if a compound such as sulfadimethoxine, which is approximately 98 per cent protein-bound, has a good clinical effect and also gives excellent results in experimental infections (5).

The protein binding is, however, reversible and it is reasonable to deduce that under the specific experimental conditions employed, the strength of the binding is of greater importance than the *extent* of the binding, and that the apparent disagreement between clinical and laboratory findings may be due to these circumstances.

The antibacterial activity of sulfonamides *in vitro* may not always be a good indication of their therapeutic value. If, however, a patient's serum

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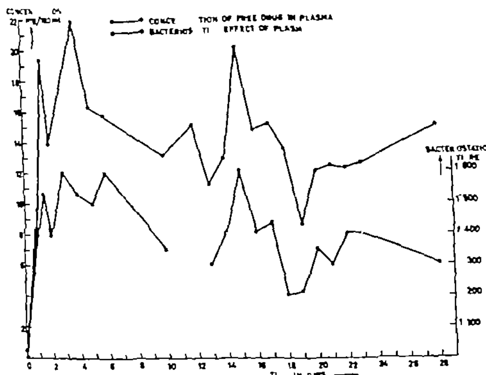


Fig. 1 Sulfadimethoxine. Correlation between serum content of non-acetylated drug and antibacterial effect. Case A. M. Varying daily doses. Bacteriostatic titer is less than 1 10 after adding p-aminobenzoic acid.

The results are shown in figs. 1 and 2. Case A.M. differs from the other cases in respect to the antibacterial titers, which are nearly twice as high at equal sulfonamide concentrations. The parallelism is otherwise apparent, as the antibacterial effect of serum varies with the chemically present sulfonamide. If a sulfonamide antagonist (p-aminobenzoic acid) is added, the antibacterial effect decreases to titers of less than 1/10 (indicated with \ on fig 1)

Secondly, sulfamethoxypyridazine was investigated in detail in 4 patients.

\ J 58 ears old female.
Mitral stenosis, pleural effusion (hydrothorax)

O N 28 years old male.
Dyspepsia.

J.K. 71 years old female.
Aplastic anemia, diabetes mellitus.

N M.K. 76 years old male.
Bronchial asthma, coronary heart disease.

Fig 3 shows the results obtained. There is a close connection between the antibacterial effect of the serum samples and the concentration of sulfamethoxypyridazine.

In the third group 4 patients were given sulfadiazine.

B.Y 22 years old female.
Acute pyelocystitis.

R.L. 63 years old male.
Coronary heart disease.

after administration of a sulfonamide has a strong antibacterial activity this must be considered a good test for the potency of that sulfonamide, whether it is protein bound or not.

We have investigated the antibacterial activity of serum and extravascular fluid in patients after administration of long acting sulfonamides, *sulfadimethoxine*, which has been stated to be 98 per cent protein bound (10) and of sulfamethoxy pyridazine which is protein bound to a lesser degree. For comparison we have used the "standard sulfonamide" sulfadiazine which is said to be about 50 per cent protein bound (10)

Material and methods

Sulfadimethoxine, sulfamethoxypyridazine and sulfadiazine have been used separately in each case. Drugs giving rise to a falsely positive Bratton and Marshall reaction have been carefully avoided. Only the free, non-acetylated sulfonamide has been determined, the acetyl-derivatives being without any antibacterial effect.

Sulfadimethoxine and sulfamethoxypyridazine have been given in an initial dose of 1 0 g followed by 0.5 g as a daily maintainance dose, whereas the dosage of sulfadiazine amounted to 1 0 g 4 times daily In 12 cases the dosage differed from this scheme. Four patients were given sulfadimethoxine, 4 were given sulfamethoxy pyridazine and 4 were given sulfadiazine. The dosages in these cases are plotted on the respective figures.

Sulfadimethoxine was tested on 22 patients, sulfamethoxypyridazine on 6 and sulfadiazine on 13

In a series of cases with edema, ascites, pleural effusion or arthritic exudates, samples from these fluids and from blood have been drawn simultaneously for comparative studies.

The protein contents of the fluids mentioned have been estimated by means of the biuret method.

The chemical determinations of sulfonamides were carried out according to a modification of the Bratton and Marshall method described by Drucy and Oesterheld (4)

Only free sulfonamide was determined, and the determinations were done in serum. The extinction values were read on an Elko II photometer (Zeiss)

The bacteriostatic effect of the serum samples was determined using the dilution method. The sera were diluted with the medium described by Adams and Roe (1) This medium contains no sulfonamide inhibitors. The serum dilutions used were as follows 1/20, 1/40, 1/80 1/100, 1/150, 1/200 1/300 1/400, 1/600 1/800 and the volume in each test tube was 1 ml.

The test culture employed was a strain of *Shigella dysenteriae III* and for inoculation an 18—20 hour culture in Adams and Roe's medium was used. This culture was diluted to give app. 200—300 bacteria in one small drop Each test tube was inoculated with this one drop of this dilution, and the tubes were incubated for 18 hours at 37° C.

The titers are given as the last dilution showing no growth.

The bacteriostatic titer of normal human sera containing no sulfonamide is with this technique 1/10 or less, and it has not been considered necessary to inactivate the sera before use.

As a check on the assay a standard solution of the sulfonamide in question was titrated in the same way as the serum samples.

All analyses have been carried out in duplicate.

Results

Comparison between the sulfonamide content and the antibacterial effect of serum

Sulfadimethoxine was investigated in detail in 4 patients

A.M. 55 years old female.
Acute cystitis, multiple sclerosis.
K.S. 84 years old female.
Peptic ulcer coronary heart disease.
J.L. 59 years old female.
Bronchopneumonia, pulmonary emphysema.
Ö J 60 years old male.
Chronic bronchitis, bronchopneumonia.

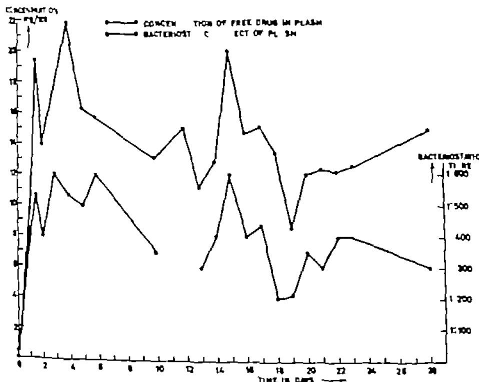


Fig. 1. Sulfadimethoxine. Correlation between serum content of non-acetylated drug and antibacterial effect. Case A. M. Varying daily doses. Bacteriostatic titer is less than 1: 10 after adding p-aminobenzoic acid

The results are shown in figs. 1 and 2. Case A.M. differs from the other cases in respect to the antibacterial titers, which are nearly twice as high at equal sulfonamide concentrations. The parallelism is otherwise apparent, as the antibacterial effect of serum varies with the chemically present sulfonamide. If a sulfonamide antagonist (p-aminobenzoic acid) is added, the antibacterial effect decreases to a titer of less than 1/10 (indicated with X on fig. 1)

Secondly sulfamethoxypyridazine was investigated in detail in 4 patients.

A.J. 58 years old female.
Mitral stenosis, pleural effusion (hydrothorax)

O.N. 28 years old male.
Dyspepsia.

J.K. 71 years old female.
Aplastic anemia, diabetes mellitus.

N.M.K. 76 years old male
Bronchial asthma, coronary heart disease.

Fig. 3 shows the results obtained. There is a close connection between the antibacterial effect of the serum samples and the concentration of sulfamethoxypyridazine.

In the third group 4 patients were given sulfadiazine.

B.Y. 22 years old female.
Acute pyelocystitis.

R.L. 63 years old male.
Coronary heart disease.

after administration of a sulfonamide has a strong antibacterial activity this must be considered a good test for the potency of that sulfonamide, whether it is protein-bound or not.

We have investigated the antibacterial activity of serum and extravascular fluid in patients after administration of long acting sulfonamides sulfadimethoxine, which has been stated to be 98 per cent protein bound (10) and of sulfamethoxy pyridazine which is protein bound to a lesser degree. For comparison we have used the standard sulfonamide sul fadiazine which is said to be about 50 per cent protein-bound (10)

Material and methods

Sulfadimethoxine, sulfamethoxypyridazine and sulfadiazine have been used separately in each case Drugs giving rise to a falsely positive Bratton and Marshall reaction have been carefully avoided Only the free, non acetylated sulfonamide has been determined the acetyl-derivatives being without any antibacterial effect.

Sulfadimethoxine and sulfamethoxypyridazine have been given in an initial dose of 1 0 g followed by 0.5 g as a daily maintainance dose, whereas the dosage of sulfa diazine amounted to 1 0 g 4 times daily In 12 cases the dosage differed from this scheme. Four patients were given sulfa dimethoxine, 4 were given sulfamethoxy pyridazine and 4 were given sulfadiazine The dosages in these cases are plotted on the respective figures.

Sulfadimethoxine was tested on 22 patients, sulfamethoxypyridazine on 6, and sulfa diazine on 13

In a series of cases with edema, ascites, pleural effusion or arthritic exudates, samples from these fluids and from blood have been drawn simultaneously for comparative studies.

The protein contents of the fluids mentioned have been estimated by means of the biuret method.

The chemical determinations of sul fonamides were carried out *according to a* modification of the Bratton and Marshall method described by Druey and Oesterheld (4)

Only free sulfonamide was determined, and the determinations were done in serum. The *extinction values* were read on an Elko II photometer (Zeiss)

The bacteriostatic effect of the serum samples was determined using the dilution method. The sera were diluted with the medium described by Adams and Roe (1) This medium contains no sulfonamide inhibitors. The serum dilutions used were as follows 1/20, 1/40, 1/80 1/100, 1/150 1/200, 1/300 1/400 1/600 1/800 and the volume in each test tube was 1 ml.

The test culture employed was a strain of Shigella dysenteriae III and for inoculation an 18—20 hour culture in Adams' and Roe's medium was used. This culture was diluted to give app 200—300 bacteria in one small drop Each test tube was inoculated with this one drop of this dilution and the tubes were incubated for 18 hours at 37 C.

The titers are given as the last dilution showing no growth.

The bacteriostatic titer of normal human sera containing no sulfonamide is with this technique 1/10 or less, and it has not been considered necessary to inactivate the sera before use.

As a check on the assay a standard solution of the sulfonamide in question was titrated in the same way as the serum samples.

All analyses have been carried out in duplicate.

Results

Comparison between the sulfonamide content and the antibacterial effect of serum

Sulfadimethoxine was investigated in detail in 4 patients

A.M. 55 years old female.
Acute cystitis, multiple sclerosis.

K.S. 84 years old female.
Peptic ulcer coronary heart disease.

J.L. 59 years old female
Bronchopneumonia, pulmonary emphysema.

Ö J 60 years old male.
Chronic bronchitis, bronchopneumonia.

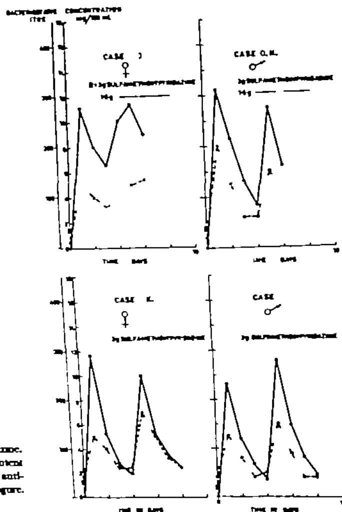
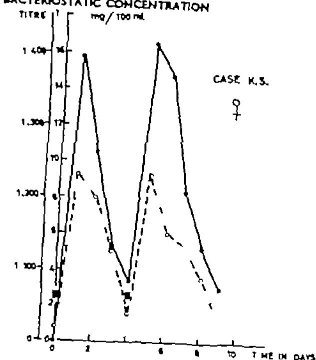
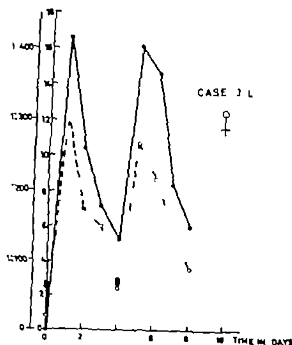


Fig 3. Sulfamethoxypyridazine. Correlation between serum content of nonacetylated drug and antibacterial effect Dosage see figure.

concentration in serum In cases B Y and R.L. the starting points on the respective graphs are not at zero as sulfadiazine had been administered to these patients before the experiment was started.

These three experiments demonstrate that during sulfonamide treatment, the antibacterial activity of serum depends on the sulfonamide concentration in serum. This is the case whether the sulfonamide in question is a conventional one (g sulfadiazine) or a long-acting sulfonamide Moreover the experiments show that the antibacterial effect resulting from equal sulfonamide concentrations is similar for the three drugs. In other words, the depot-sulfonamides do not exhibit any lower bacteriostatic titers than sulfadiazine. In case A.M. where a long-acting sulfonamide was given, we even find considerably higher antibacterial titers than in the other cases, although the experimental conditions were identical.





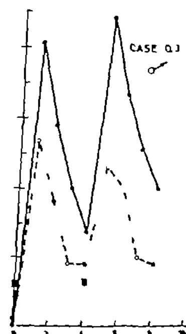


Fig 2 Sulfadimethoxine. Correlation between serum content of non-acetylated drug and antibacterial effect. Black squares 2 g sulfadimethoxine orally

E.B. 54 years old male.
Acute pneumonia, stomatitis.
L.F 58 years old male.
Mitral stenosis, congestive heart failure.

The results are presented in fig 4 and as in the first two groups, there is also here a close parallelism between the antibacterial effect and the sulfonamide

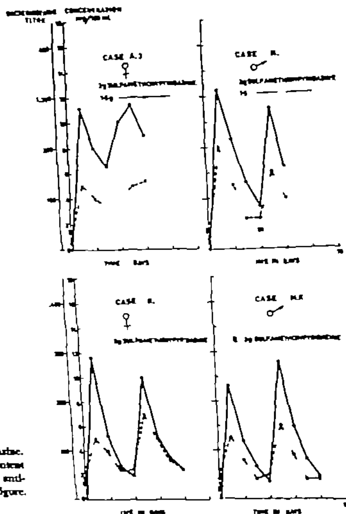


Fig. 3 Sulfamethoxypyridazine. Correlation between serum content of nonacetylated drug and antibacterial effect. Dosage see figure.

concentration in serum. In cases B.Y and R.L. the starting points on the respective graphs are not at zero as sulfadiazine had been administered to these patients before the experiment was started.

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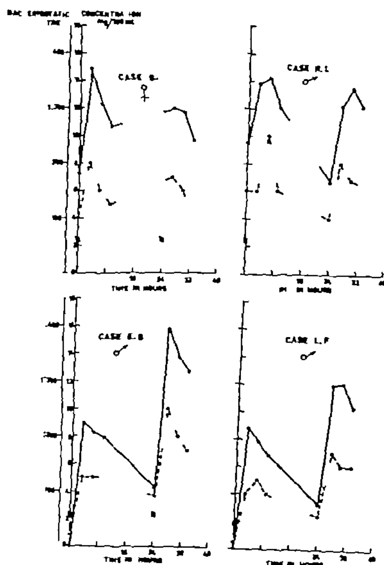


Fig. 4 Sulfadiazine. Correlation between serum content of non-acetylated drug and antibacterial effect. Black squares 4 g sulfadiazine orally

Antibacterial effect of sulfadimethoxine in serum and in protein free diluent

Sixty nine serum samples from 21 patients have been investigated Case A.M. has not been taken into account here, as the antibacterial titers from this patient for unknown reasons are much higher than all others encountered For comparison the antibacterial effect of sulfadimethoxine in a protein free dilution has also been determined. The diluent used was the same as that used for the serum dilutions (see above)

Fig 5 gives the results.

The experiment shows that under the present experimental conditions, the antibacterial effect of equal amounts of sulfadimethoxine is independent of whether protein is present or not.

Comparison of antibacterial effect of sulfadiazine and of sulfamethoxypyridazine

This investigation includes 36 serum samples from 6 patients treated with sulfamethoxypyridazine, and 55 serum samples from 13 patients treated with sulfadiazine. The results are given in fig 6 It is evident that the antibacterial

Fig. 5. Sulfadimethoxine. Correlation between concentration of drug and antibacterial effect in serum and in protein-free diluent.

effect of equal amounts of sulfamethoxypyridazine and sulfadiazine is the same under the conditions applied.

Sulfadimethoxine in extravascular fluids

One of the main questions in all sulfonamide treatment is the transfer of the drug from the blood to the extravascular fluid. In order to illuminate this point, we have investigated parallelly the concentration and antibacterial effect of sulfadimethoxine in serum and in extravascular fluids. Blood and fluid samples were withdrawn at the same time and 12 patients with various complaints were examined.

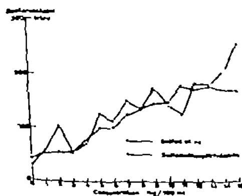


Fig. 6. Relationship between serum concentration and bacteriostatic effect.

Table I Sulfadimethoxine in serum and in extravascular exudates. Comparison between concentration, antibacterial effect and protein content

Patient	Exudate examined	Extravascular exudates			Serum
		Protein content (g/100 ml)	Antibacterial effect	Non-acetylated sulfonamide (mg/100 ml)	Non-acetylated sulfonamide (mg/100 ml)
G B.	Arthritic	4.9	300	12.0	11.9
K. H.	—	9 4	300	11 1	11.6
J T	Ascites	2.0	200	9 4	16.3
K. H.	Arthritic	6.3	150	9.3	9.7
H. H.	Pleural	4.0	250	9.0	12.9
K. H.	Arthritic	6.5	200	8.8	12.8
B. V	—	5.9	150	8.5	10.6
B. K.	Pleural	4.8	100	8.3	7.9
H. H.	—	3.8	300	8.2	?
B. K.	—	5.0	100	7.3	10.7
K. H.	Arthritic	6.4	150	7 0	11.3
K. H.	—	5.0	150	6.8	11 1
L. E.	Ascites	4.0	150	6.7	9.7
I O	Edema	2.5	60	6.0	10.4
B. K.	Pleural	4.8	150	5.6	12.1
L. E.	Ascites	3.0	100	5.6	?
S M.	Ascites	1.3	20	3.4	7.3
J T	—	2.5	30	2.9	6.9
O B.	—	1.5	60	2.9	8.3
A. O	Pleural	3.4	<20	2.6	10.6
K. E.	Edema	0.8	60	< 2.2	8.8
K. E	—	0.8	80	< 2..	8.8
L. O.	—	2.4	<20	2.1	8.5
J T	Ascites	2.2	20	1.9	5.1
K. E.	Edema	0.8	40	1.8	7.2
S. M.	Ascites	1 1	10	1 4	4.9
S. M.	—	1 1	<20	0.8	4.7

Inverse values of the titers.

K.H. 36 years old male.
Purulent arthritis (knee-joint)

B.V 62 years old female.
Chronic bronchitis, chronic poly arthritis.

G B. 28 years old male.
Reiter's disease, articular exudates.

H.H. 83 years old female.
Pulmonary cancer with pleural effusion.

O B. 69 years old male.
Myelofibrosis, diabetes mellitus, ascites.

B.K. 55 years old female.
Pulmonary cancer with pleural effusion.

A.O 61 years old male.
Pulmonary cancer with pleural effusion.

K.E. 75 years old female.
Myocardial infarction, congestive heart disease.

I O 74 years old female.
Hypertension, congestive heart disease.

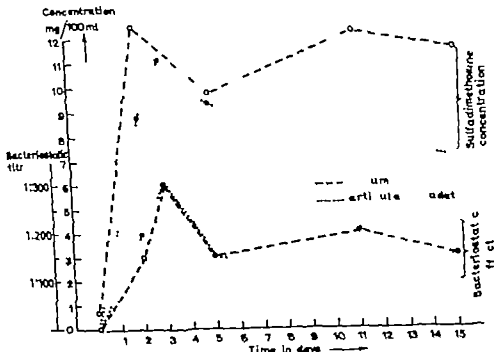


Fig. 7 Sulfadimethoxine. Correlation between amount of drug and antibacterial effect in serum and in articular exudate.

J T 78 years old male. Cirrhosis of the liver ascites.
I.E. 66 years old female. Cancer with ascites.
S.M. 59 years old female. Cirrhosis of the liver ascites, edema.

The results are given in table I The table shows a very good correlation between sulfonamide concentration and antibacterial effect in the extravascular fluids, independent of the protein content in these.

One of the patients, K.H., has been investigated with particular consideration to the antibacterial activity of the very protein-rich articular exudate (fig 7) The figure shows that the bacteriostatic effect under the present experimental conditions is good (titers between 150—300) regardless of the high protein content (table I case K.H.) It is also apparent that antibacterial titer and sulfonamide concentration of the articular exudate parallel the titer and sulfonamide concentration of serum.

Discussion

Under the experimental conditions applied during the present investigation, it appears that the antibacterial effect of long-acting sulfonamides, sulfadimethoxine and sulfamethoxypyridazine, is equal to that of sulfadiazine. In all experiments with serum this antibacterial effect is directly dependent on the sulfonamide concentration As the difference in protein-binding for these sulfonamides is great, it is reasonable to

assume that this binding is of minor importance as concerns the antibacterial activity in serum. The fact that it is possible under special circumstances (9) to demonstrate a decrease in antibacterial activity of sulfonamides *in vitro* due to protein binding is a different matter The present investigation has been planned with the intention of obtaining conditions as near as possible to those encountered *in vivo*.

Furthermore, the experiments show that the particular sulfonamide which according to the literature is bound to protein to the highest degree of those discussed here, namely sulfadimethoxine, exhibits the same antibacterial effect from a given sulfonamide concentration, whether this is present in serum or in a protein free diluent. This is also in opposition to several investigators (2 3 9) who state that the antibacterial effect is considerably decreased in the presence of protein Their investigations have however been performed under very specific conditions, which in many cases are far from those *in vivo* This is of great theoretical interest, but may hold minor therapeutic implications. Our investigations seem to indicate that the sulfonamides possess a greater affinity to bacteria than to the serum proteins, at any rate with the employed test microbe We will not exclude the possibility that other microbes may show a different behaviour

In studying the transfer of sulfonamides from blood to extravascular exudates, we found that sulfonamide (sulfadimethoxine) does penetrate into the extravascular fluids and preferably where the protein content is high. It therefore appears that the sulfonamide concentration one obtains in the extravascular fluid depends not only on the serum concentration but also on the protein content in the fluid. In cases of high sulfonamide concentrations in serum, and ample amounts of protein in the extravascular fluid it is possible to obtain fairly high sulfonamide concentrations also in the latter

It is important to emphasize our finding that a sulfonamide after diffusing into extravascular fluid has the same antibacterial effect as in serum. This is evident also when the protein content of the fluid is high. In other words, the experiments show that a long-acting sulfonamide (sulfadimethoxine) under the employed experimental conditions diffuses into extravascular fluids and retains its antibacterial effect

Summary

It has long been maintained that the protein bound fraction of sulfonamides has no antibacterial activity Since the introduction of long acting sulfonamides, which are protein bound up to a degree of 98 per cent it has been difficult to explain that these sulfonamides have a good clinical effect and also give excellent results in experimental infections.

The authors examined the sulfonamide concentration and antibacterial activity of serum after administration of two long acting sulfonamides, sulfadimethoxine and sulfamethoxypyridazine, and for comparison the "standard sulfonamide" sulfadiazine. A comparison between sulfonamide concentration and antibacterial activity of extravascular exudates was also made

It was found that the antibacterial activity of serum after administration of long-acting sulfonamides was equal to that after administration of sulfadiazine. In all experiments there was a close

connection between the sulfonamide concentration and antibacterial activity regardless of the protein-binding" The same close correlation was also found with regard to various exudates. It is reasonable to assume that the protein binding is of minor importance with regard to the antibacterial activity and also that the strength of the binding is more important than the extent of the binding.

In studying the transfer of sulfonamides from blood to exudates, it was found that a long-acting sulfonamide (sulfadimethoxine) penetrated into extravascular fluids and especially where the protein content was high. In exudates the same close correlation and antibacterial activity was found as in serum.

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Studies on the Effect of Nicotinic Acid on Catecholamine Stimulated Lipolysis in Adipose Tissue in Vitro

By

LARS A. CARLSON

It was recently demonstrated by Carlson and Orö (2) that treatment of dogs with nicotinic acid inhibited the increase in plasma free fatty acids (FFA) normally elicited by norepinephrine infusion, while the blood pressure response was unchanged. It was suggested that this effect of nicotinic acid might be due to a direct blocking in adipose tissue of the FFA release from this organ. This hypothesis has been studied and confirmed in this paper. A reduced output of FFA from adipose tissue might well be the explanation to the hitherto unknown mechanisms by which nicotinic acid lowers plasma cholesterol and glycerides (2). It is known that nicotinamide does not affect the plasma cholesterol and glyceride concentration, while nicotinic acid exerts a lowering action on the plasma lipids (1, 7). It was considered also to be of interest to study the effect of these two compounds on adipose tissue.

Submitted for publication December 12, 1962.

Methods

Incubation of adipose tissue in vitro

Epididymal fat pads from rats were used for the incubation studies. Rats weighing about 200—250 g and fasted for 20—24 hours were anesthetized with intraperitoneal Nembutal, the epididymal fat pads were excised and cut into pieces and divided between the incubation flasks in such a way that each flask contained pieces from each rat and from each part of the fat pad. The incubation medium consisted of 2 % human albumin in Krebs-Ringer-phosphate buffer pH 7.4. The incubations were performed for one hour at 37° C with gentle shaking. Additions to the medium were done at zero time.

Analytical methods

After incubation samples were taken off from the medium and immediately extracted for determination of FFA according to Dole (3) and also immediately precipitated with perchloric acid for the enzymatic determination of glycerol according to Wieland (9). Addition of the different drugs studied was found not to interfere with the methods used. The values have been calculated as micromoles liberated to the medium per gram adipose tissue and hour of incubation.

Kindly supplied by A.B. Kabi, Stockholm, Sweden.

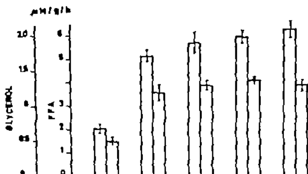


Fig. 2. The effect of nicotinamide on the norepinephrine stimulated release of glycerol and FFA from adipose tissue incubated *in vitro*. Each bar represents the mean of five incubation flasks. I represents standard error of the mean.

Addition to the medium	Norepinephrine γ/ml	0		0.2		0.2		0.2		0.2
	Nicotinamide M	0		0		10^{-8}		10^{-6}		10^{-4}

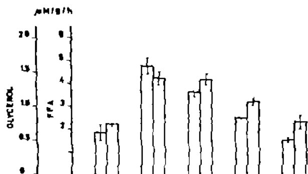


Fig. 3. The effect of nicotinic acid on the norepinephrine stimulated release of glycerol and FFA from adipose tissue incubated *in vitro*. Each bar represents the mean of five incubation flasks. I represents standard error of the mean.

Addition to the medium	Norepinephrine γ/ml	0		0.2		0.2		0.2		0.2
	Nicotinic acid M	0		0		10^{-8}		10^{-6}		10^{-4}

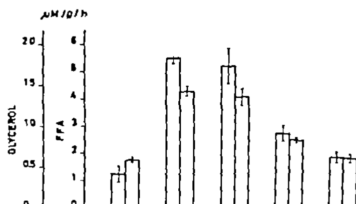
Discussion

These results have given clearcut evidence that nicotinic acid blocks the direct stimulating effect norepinephrine exerts on the release of FFA from adipose tissue. Catecholamines increase the amount of circulating FFA by stimulating the output of FFA from adipose tissue (4). Evidence is available that this increased output is due to an enhanced lipolysis of the stored triglycerides in adipose tissue. Thus it has been shown that not only is the amount of FFA released increased but also the amount of glycerol (6). Glycerol is, of course, the final breakdown product during lipolysis and the glycerol once liberated does not appear to be reesterified in adipose tissue as the liberated fatty

Table I. Effect of the addition of nicotinic acid, nicotinamide and nicotinuric acid at a concentration of 10^{-4} M on the basal release of glycerol and FFA from adipose tissue incubated in vitro in Krebs Ringer buffer with 2 % albumin

Exp. no.	1		2		3	
	No addition	Nicotinic acid	No addition	Nicotinamide	No addition	Nicotinuric acid
Glycerol μM/g/h	0.43 ± 0.02	0.40 ± 0.03	0.69 ± 0.04	0.67 ± 0.02	0.66 ± 0.08	0.54 ± 0.09
FFA μE/g/h	1.66 ± 0.08	1.78 ± 0.15	1.19 ± 0.10	0.95 ± 0.10	2.24 ± 0.04	2.38 ± 0.30

Mean value and standard error of the mean from five incubations.



Addition to the medium	Norepinephrine γ/ml	0		0.2		0.2		0.2		0.2
	Nicotinic acid M	0		0		10^{-6}		10^{-5}		10^{-4}

Fig. 1. The effect of nicotinic acid on the norepinephrine stimulated release of glycerol and FFA from adipose tissue incubated *in vitro*. Each bar represents the mean of five incubation flasks. I represents standard error of the mean.

Results

Effect on basal release from adipose tissue

In table I the results are given on the effect of addition of nicotinic acid, nicotinamide and nicotinuric acid at a concentration of 10^{-4} M on the release of glycerol and FFA. As can be seen, the addition of these drugs did not have any significant influence on the basal release from adipose tissue.

Effect on the norepinephrine stimulated release from adipose tissue

The results from three different experiments with the addition of nicotinic acid, nicotinamide and nicotinuric acid respectively are given in figs. 1, 2 and 3. It is seen that the addition of norepinephrine to the incubation medium considerably increased the release of glycerol as well as of FFA. Nicotinic acid at a concentration of 10^{-5} M significantly reduced the norepinephrine stimulated release of glycerol and FFA and this effect was still more pronounced at the concentration 10^{-4} M. Fig. 2 shows that nicotinamide did not influence the norepinephrine stimulated release. Nicotinuric acid, on the other hand, inhibited the catecholamine induced release of glycerol and FFA as is evident from fig. 3.

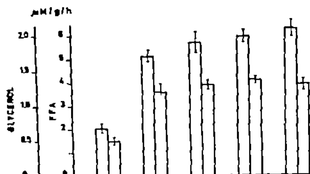


Fig. 2. The effect of nicotinamide on the norepinephrine stimulated release of glycerol and FFA from adipose tissue incubated *in vitro*. Each bar represents the mean of five incubation flasks. I represents standard error of the mean.

Addition to the medium										
	Norepinephrine μg/ml	0		0.2		0.2		0.2		0.2
	Nicotinamide M	0		0		10^{-6}		10^{-5}		10^{-4}

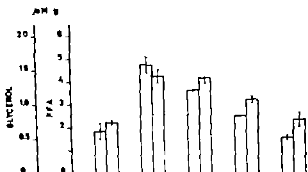


Fig. 3. The effect of nicotinic acid on the norepinephrine stimulated release of glycerol and FFA from adipose tissue incubated *in vitro*. Each bar represents the mean of five incubation flasks. I represents standard error of the mean.

Addition to the medium										
	Norepinephrine μg/ml			0.2		0.2		0.2		0.2
	Nicotinic acid M			0		10^{-6}		10^{-5}		10^{-4}

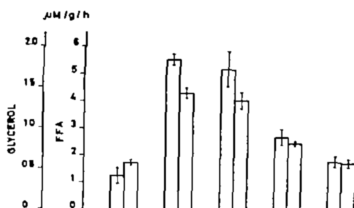
Discussion

These results have given clearcut evidence that nicotinic acid blocks the direct stimulating effect norepinephrine exerts on the release of FFA from adipose tissue. Catecholamines increase the amount of circulating FFA by stimulating the output of FFA from adipose tissue (4). Evidence is available that this increased output is due to an enhanced lipolysis of the stored triglycerides in adipose tissue. Thus it has been shown that not only is the amount of FFA released increased but also the amount of glycerol (6). Glycerol is, of course, the final breakdown product during lipolysis and the glycerol once liberated does not appear to be reesterified in adipose tissue as the liberated fatty

Table I. Effect of the addition of nicotinic acid, nicotinamide and nicotinuric acid at a concentration of 10^{-4} M on the basal release of glycerol and FFA from adipose tissue incubated in vitro in Krebs Ringer buffer with 2 % albumin

Exp. no.	1		2		3	
	No addition	Nicotinic acid	No addition	Nicotinamide	No addition	Nicotinuric acid
Glycerol µM/g/h	0.43 ± 0.02	0.40 ± 0.03	0.69 ± 0.04	0.67 ± 0.02	0.66 ± 0.08	0.54 ± 0.09
FFA µE/g/h	1.66 ± 0.08	1.78 ± 0.15	1.19 ± 0.10	0.95 ± 0.10	2.24 ± 0.04	2.38 ± 0.30

Mean value and standard error of the mean from five incubations.



Addition to the medium	Norepinephrine γ/ml	0		0.2		0.2		0.2		0.2
	Nicotinic acid M	0		0		10^{-6}		10^{-5}		10^{-4}

Fig. 1. The effect of nicotinic acid on the norepinephrine stimulated release of glycerol and FFA from adipose tissue incubated in vitro. Each bar represents the mean of five incubation flasks. I represents standard error of the mean.

Results

Effect on basal release from adipose tissue

In table I the results are given on the effect of addition of nicotinic acid, nicotinamide and nicotinuric acid at a concentration of 10^{-4} M on the release of glycerol and FFA. As can be seen, the addition of these drugs did not have any significant influence on the basal release from adipose tissue.

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From the Medical Department II (Head Hans Heckscher M. D.), Municipal Hospital, Copenhagen, Denmark

The Influence of Arterial Blood Gases and the Mental State on the Activity Pattern of the Diaphragm and Some Muscles of the Trunk and Neck in Patients with Asthma and Emphysema

By

PALLE GRØNBÆK, ERIK MOLTKE and ARNE P. SKOUBY

In a previous paper the abnormal activity pattern of the diaphragm and some muscles of the neck and trunk in patients with chronic asthma and also emphysema was analysed by electromyographic techniques. An *increased permanent activity* of the superficial muscles was demonstrated, while only inspiratory activity of the diaphragm was the usual finding. In the superficial muscles inspiratory electromyographic activity usually began and expiratory activity ended simultaneously at the onset of electromyographic (inspiratory) activity of the diaphragm. This phenomenon is called *synchronism* in the following paper. Synchronization was seldom found for the times at which inspiratory activity disappeared or expiratory activity appeared in the muscles.

In normal individuals a similar pattern with an increased permanent activity only of the superficial muscles and with "synchronism" was not obtained during forced breathing, breathing against artificial external resistances or during rebreathing oxygen or air for up to five minutes. Thus, other factors than those investigated in these experiments of short duration with normal subjects must be the reason for the characteristic muscular behaviour found in the patients (3).

The present study was performed in order to elucidate whether the activity pattern of the patients is related to the nature and degree of the respiratory insufficiency and/or to the mental state.

Methods

The muscular activity was estimated by electromyography and the degree of respiratory insufficiency by arterial blood analyses. The influences of the nature of the respiratory disorder and of the mental state was estimated from the results obtained in selected groups of patients, and in normal subjects breathing with an increased dead space against artificial external resistances.

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acids can be (5) Glycerol can thus be assumed to be a better direct indicator of the lipolytic process than FFA. As is seen in fig 1 norepinephrine stimulated the glycerol release proportionately more than the FFA release. The inhibition of this stimulated release by nicotinic acid *affects glycerol as well as FFA*, indicating a direct inhibition on the lipolysis. The mechanism of this effect is unknown However it is known that there exists an epinephrine sensitive lipase in adipose tissue (8) and nicotinic acid might have an effect on this enzyme when it is catecholamine activated In this connection it is interesting to note that nicotinic acid had no effect on the basal, noncatecholamine stimulated release. The reason for this is unknown among possible explanations are that either catecholamines are of no greater importance *for the basal release or that nicotinic* acid cannot block the effect of adipose tissue bound catecholamines. Furthermore this suggests that nicotinic acid acts as a true adrenolytic agent and is not only inhibiting the lipase.

Nicotinuric acid showed a blocking *effect on the norepinephrine* induced lipolysis similar to nicotinic acid Both these substances are effective in lowering plasma lipid levels. Nicotinamide, on the other hand which is ineffective with regard to lowering of blood lipids, did not show this metabolic sympathicolytic effect. This lends further support to the hypothesis, that one possible way for lowering blood lipids is by decreasing the influx of the substrate FFA to the liver simply by reducing its output from adipose tissue.

Summary

The effect was studied of nicotinic acid nicotinamide and nicotinuric acid on the basal release as well as on the norepinephrine stimulated release of glycerol and free fatty acids from adipose tissue *in vitro*

Neither substance significantly affected the basal release from adipose tissue of glycerol or free fatty acids (FFA)

Nicotinic acid and nicotinuric acid both at a concentration of 10^{-4} M significantly reduced the *norepinephrine stimu*lated release of glycerol as well as of FFA. Nicotinamide had no effect on this stimulated release.

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Methods

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Table I. Number of patients with emphysema and asthma included in the study. Distribution according to sex and age

Diagnosis		Sex	Age			Total	
			< 40	40–60	> 60	♀	♂
Acute intermittent asthma		♀	3	3	0	6	
		♂	1	2	3		6
Chronic asthma	Nervous	♀	*0*	*9*	2	11	
		♂	0	4	0		4
	Non-nervous	♀	*0*	*1*	0	*1*	
		♂	0	3	4		7
Chronic emphysema	Nervous	♀	0	2	0	2	
		♂	*0*	5	3		8
	Non-nervous	♀	0	2	0	2	
		♂	0	8	11		19
		Total	4	39	23	22	44

Electromyography

A DISA electromyograph was used as the recording instrument. Paper speed 5 cm/sec.

The activity from the diaphragm was recorded via the esophagus by a bipolar electrode constructed in collaboration with Mrs. Annelise Rosenfalck, Institute of Neurophysiology Copenhagen.

Activity from the muscles of the neck and trunk was recorded by bipolar surface electrodes made from tin. The electrodes were fastened by sticking-plaster. The skin resistance was reduced by electrode paste. A detailed description of the electromyographic technique has been given previously (3).

The examination always included the diaphragm, *m. obliquus externus abdominis*, m. serratus magnus, m. pectoralis major, m. sternocleidomastoideus, m. trapezius, m. regio supra- and infraspinata, m. latissimus dorsi and m. sacrospinalis. All recordings were performed on subjects standing at ease.

For the recording of the activity from the diaphragm an amplification corresponding to $10\,\mu V = 1$ mm was used. To avoid base line swings frequencies less than 20 Hz were filtered off. From the superficial muscles electromyograms were obtained with three amplifications corresponding to 30, 15 and $10\,\mu V = 1$ mm in order to estimate the degree of activity. Each record included two superficial muscles and the diaphragm.

In a previous study (3) the inspiration phases usually corresponded fairly well to the activity of the diaphragm. When phasic activity of this muscle appeared during expiration this was easily detected by observation of the subject and the oscilloscopes. Furthermore a characteristic electromyographic pattern disclosed the phenomenon. It was therefore decided to avoid the possible influence caused *by the use of a marking aggregate and to* estimate the respiration phases only from the beginning and end of the inspiratory diaphragmatic activity. This was found to be possible throughout the study.

In the following the terms inspiratory and expiratory activity include discharges with a maximum in the inspiration and the expiration phase respectively. The term permanent activity is used when no zero level was seen during the respiration cycle.

The degree of permanent activity in the superficial muscles was estimated from the amplification necessary *for visualizing* the activity, as no device for measurement of the mean voltage of the electromyogram was at our disposal. For indisputable activity obtained with the amplifications corresponding to 30, 15 and $10\,\mu V = 1$ mm, scores of 3,

Table II. Values for calculated permanent activity, oxygen saturation, pH and pCO_2 in patients with severe dyspnea caused by other disorders than emphysema and asthma

Diagnosis	Sex	Age	Calculated permanent activity	Oxygen saturation in %	pH	pCO
Pu[illegible]	♂	44	9.5	87	7.32	52
Bronch. carcinoma	♂	72	6	89.5	7 44	30
Cardiac insufficiency	♂	72	6.5	94	7 48	33
	♂	63	10.5	96	7.53	37

1.5 and 1 point respectively were given. For dubious activity recorded with the highest degree of amplification used (10 µV = 1 mm) 0.5 point was given. The value obtained by addition of the highest scores given for the eight areas is called *calculated permanent activity* in the following.

Control experiments were performed on seven males 22—46 years of age without cardiorespiratory disease or muscular complaints. The *calculated permanent act ity in these normal subjects ranged from 0—2.5 points* with a mean value of 1 point.

Arterial blood analyses

Arterial blood was obtained immediately following the electromyographic examination. I most experiments the blood was drawn through Seldinger stopcock mounted on Polythen catheter placed in the brachial artery 2—3 hours before the experiment. Two heparinized syringes were filled with blood, placed on a bed of ice cubes and sent to the department of clinical chemistry for analysis.

The pH was measured by means of a pH meter (Radiometer) at 37° C and after thorough mixing. The oxygen saturation was measured by the Brinckmann reflectometric method. *The total carbon dioxide content* was determined by Conw y microdiffusion method and *the carbon dioxide tension* was calculated by means of the Henderson-Hasselbalch equation. All determinations were performed in duplicate on blood from both syringes.

The reliability of the determinations was tested from measurements performed on perfectly uniform samples labelled with different names. Twenty-nine samples from ten different pools ere analysed. From the maximal differences between values for oxygen saturation, pH and total carbon dioxide content found for each pool the verage maximal difference and the standard deviation for the series was calculated to be 1 1 ± 1.3 % 0.015 ± 0.011 and 1 63 ± ± 1 7 mM.

Material

The study included 66 *patients suffering from emphysema with or without asthma*. 12 with acute intermittent asthma were examined during an acute attack, while 23 with chronic asthma and also emphysema, and 31 patients with emphysema but without asthma were ex amined in a steady state of disease The patients of the two subgroups w th chronic respiratory disease were also classified before the test as either nervous or not nervous depending on their behaviour and the results of interviews. Details of sex and age are given in table I

Dyspnea patients w thout emphysema and asthma included one case of pulmonary fibrosis, one case of lung cancer nd two cases with congestive cardiac disease. All of these four patients suffered from severe orthopnea (table II)

Two normal subjects were examined during breathing with an increase of the dead space of 1 5 l against an external resistance with an opening diameter of 3—4 mm (2)

Results

Factors examined with a view to their importance for the occurrence of calculated permanent activity in patients with emphysema and asthma

For each patient the calculated permanent activity and oxygen saturation are given in fig 1 A. The symbols in-

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For each patient the calculated permanent activity and oxygen saturation are given in fig. 1 A. The symbols in-

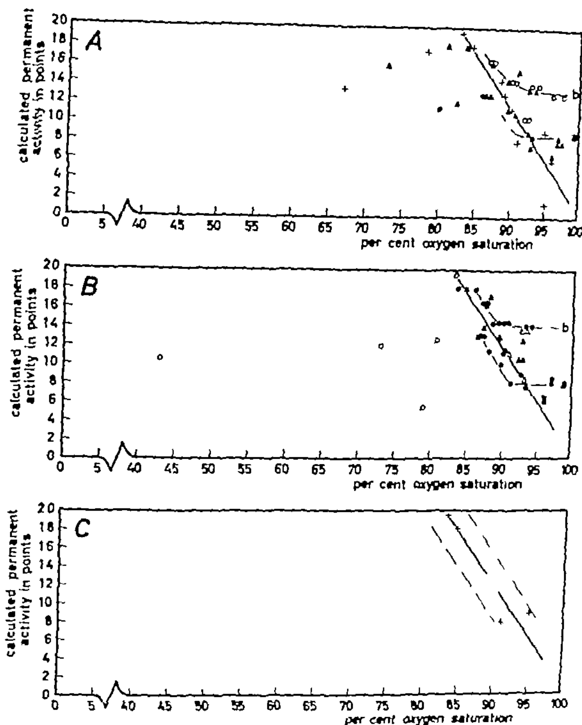


Fig 1 A. Values for calculated permanent activity in points (ordinate) and arterial oxygen saturation (abscissa) in acut intermittent asthma (+) non-nervous (●) and nervous patients with chronic asthma (○) and non-nervous (▲) and nervous patients with chronic emphysema (△)

B. Values as in 1 A. The symbols indicate alkalosis (△) hypercapnia (○) and other conditions (●)

C. Values from cases with acute asthma given separately

dicate the subgroups in question. The influence of alkalosis and of hypercapnia on the distribution can be estimated from fig 1 B. In this figure the same material as in fig 1 A is presented with symbols showing whether the values were obtained during alkalosis ($pH \geq 7.44$) hypercapnia ($pH < 7.44$ $pCO_2 \geq 46$ mm Hg) or other conditions ($pH < 7.44$ $pCO_2 < 46$ mm Hg.

The influence of the mental state is estimated from the distribution of values from patients with chronic respiratory disease. A distribution in two populations corresponding fairly well to the classification in non-nervous and nervous patients is seen in fig 1 A (dotted lines a and b) From fig. 1 B is seen that this distribution becomes more clear-cut when cases with alkalosis are excluded.

Statistical analysis by means of the t-test demonstrated that the difference between the mean values for calculated permanent activity in nervous and non-nervous subjects were

1) Highly significant a) for patients with an oxygen saturation > 90 per cent and b) for patients with an arterial $pH < 7.44$ ($p < 0.001$)

2) Significant for the patients with an oxygen saturation of 77—88 per cent ($p < 0.01$)

3) Not significant for patients with an arterial $pH > 7.44$

The influence of oxygen saturation is estimated from the distribution of values from patients with acute asthma (given separately in fig 1 C) and chronic respiratory disease (fig 1 A and 1 B)

It is seen from fig 1 C that calculated permanent activity increases with decreasing oxygen saturation until a maximum is reached at an oxygen saturation of 85 per cent. A high degree of negative correlation exists ($r = -0.94$ $y = -1.13 \times + 113.5$ $S_y = 1.8$) The regression line is given in fig 1 A—C, in C with control limits placed at two standard errors of estimate Sy on either side of the regression line.

In the only two patients with acute asthma and an oxygen saturation below 83 per cent the calculated permanent activity was below the maximum value for the group

The influence of oxygen saturation on the calculated permanent activity in the subgroups with chronic respiratory disease can be estimated from fig 1 A and 1 B.

In the two populations corresponding fairly well to non-nervous and nervous subjects no influence of oxygen saturation can be demonstrated down to 90—92 per cent saturation. In both populations a further decrease is associated with an increase in calculated permanent activity until a maximum is reached at about 85 per cent oxygen saturation The observations at still lower oxygen saturations are too few to allow of any definite information concerning the correlation between oxygen saturation and the calculated permanent activity So it is not possible to determine whether the maximum values are maintained or a decrease is taking place. The variations found are not due to alkalosis or hypercapnia (fig 1 B) They were not statistically significant when estimated by the t test, possibly due to the few observations at the varying degrees of oxygen saturation.

The influence of the different respiratory disorders can also be estimated from the figure. Apart from the different distribution with regard to oxygen saturation in acute asthma and in chronic respiratory disease no influence can be detected. It is noteworthy that the distribution in the

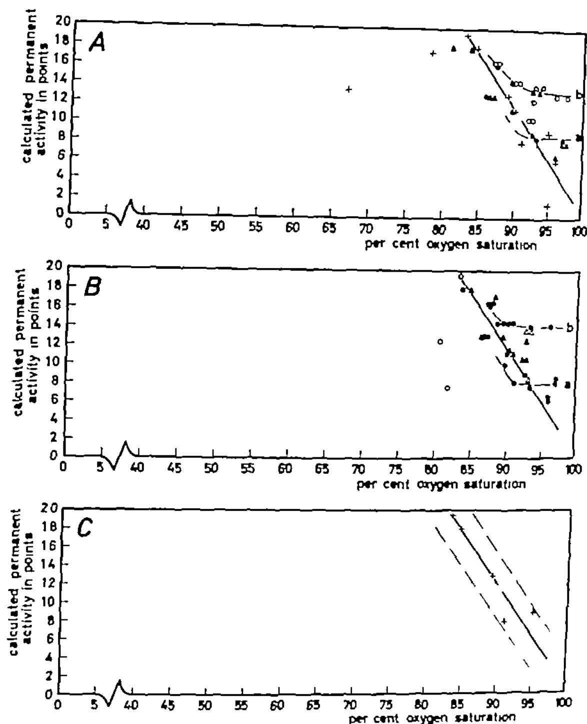


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It is seen from fig 1 C that calculated permanent activity increases with decreasing oxygen saturation until a maximum is reached at an oxygen saturation of 83 per cent. A high degree of negative correlation exists ($r = -0.94$ $y = -1.13x + 113.5$ $S_y = 1.8$) The regression line is given in fig 1 A—C, in C with control limits placed at two standard errors of estimate S_y on either side of the regression line.

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The influence of oxygen saturation on the calculated permanent activity in the subgroups with chronic respiratory disease can be estimated from fig 1 A and 1 B.

In the two populations corresponding fairly well to non nervous and nervous subjects no influence of oxygen saturation can be demonstrated down to 90—92 per cent saturation In both populations a further decrease is associated with an increase in calculated permanent activity until a maximum is reached at about 85 per cent oxygen saturation. The observations at still lower oxygen saturations are too few to allow of any definite information concerning the correlation between oxygen saturation and the calculated permanent activity So it is not possible to determine whether the maximum values are maintained or a decrease is taking place The variations found are not due to alkalosis or hypercapnia (fig. 1 B) They were not statistically significant when estimated by the t test possibly due to the few observations at the varying degrees of oxygen saturation.

The influence of the different respiratory disorders can also be estimated from the figure. Apart from the different distribution with regard to oxygen saturation in acute asthma and in chronic respiratory disease no influence can be detected. It is noteworthy that the distribution in the

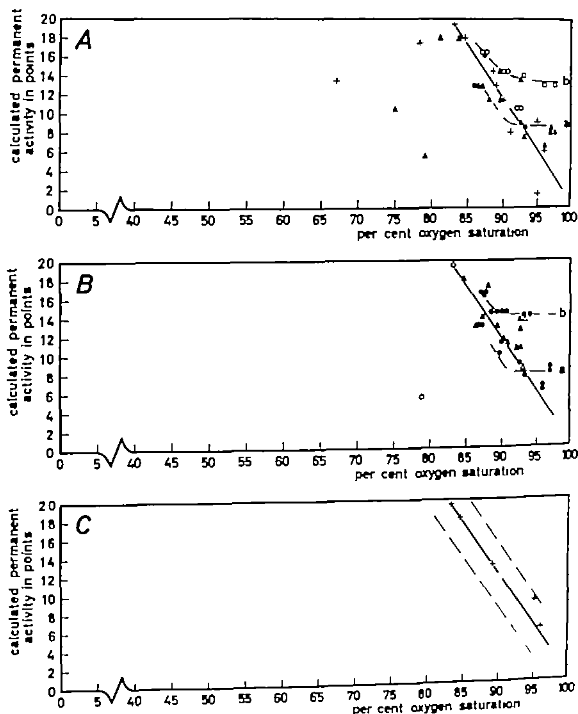


Fig 1 A. Values for calculated permanent activity in points (ordinate) and arterial oxygen saturation (abscissa) in acute intermittent asthma (+) non-nervous (●) and nervous patients with chronic asthma (○) and non-nervous (▲) and nervous patients with chronic emphysema (△)

B. Values as in 1 A. The symbols indicate alkalosis (△) hypercapnia (○) and other conditions (●)

C. Values from cases with acute asthma given separately

For further explanation: see text.

dicate the subgroups in question. The influence of alkalosis and of hypercapnia on the distribution can be estimated from fig 1 B. In this figure the same material as in fig 1 A is presented with symbols showing whether the values were obtained during alkalosis (pH $\gtrsim$ 7 44) hypercapnia (pH < 7 44 pCO_2 $\gtrsim$ 46 mm Hg) or other conditions (pH < 7 44 pCO_2 < 46 mm Hg

The influence of the mental state is estimated from the distribution of values from patients with chronic respiratory disease A distribution in two populations corresponding fairly well to the classification in non-nervous and nervous patients is seen in fig 1 A (dotted lines a and b) From fig. 1 B is seen that this distribution becomes more clear-cut when cases with alkalosis are excluded.

Statistical analysis by means of the t-test demonstrated that the difference between the mean values for calculated permanent activity in nervous and non nervous subjects were

1) Highly significant a) for patients with an oxygen saturation > 90 per cent and b) for patients with an arterial pH < 7 44 (p < 0.001)

2) Significant for the patients with an oxygen saturation of 77—88 per cent (p < 0.01).

3) Not significant for patients with an arterial pH > 7 44

The influence of oxygen saturation is estimated from the distribution of values from patients with acute asthma (given separately in fig. 1 C) and chronic respiratory disease (fig 1 A and 1 B)

It is seen from fig 1 C that calculated permanent activity increases with decreasing oxygen saturation until a maximum is reached at an oxygen saturation of 83 per cent. A high degree of negative correlation exists (r = — 0 94 y = — 1 15 x + 113.5 S_y = 1.8) The regression line is given in fig 1 A—C, in C with control limits placed at two standard errors of estimate S_y on either side of the regression line

In the only two patients with acute asthma and an oxygen saturation below 83 per cent the calculated permanent activity was below the maximum value for the group.

The influence of oxygen saturation on the calculated permanent activity in the subgroups with chronic respiratory disease can be estimated from fig 1 A and 1 B.

In the two populations corresponding fairly well to non nervous and nervous subjects no influence of oxygen saturation can be demonstrated down to 90—92 per cent saturation. In both populations a further decrease is associated with an increase in calculated permanent activity until a maximum is reached at about 85 per cent oxygen saturation The observations at still lower oxygen saturations are too few to allow of any definite information concerning the correlation between oxygen saturation and the calculated permanent activity So it is not possible to determine whether the maximum values are maintained or a decrease is taking place. The variations found are not due to alkalosis or hypercapnia (fig 1 B) They were not statistically significant when estimated by the t test, possibly due to the few observations at the varying degrees of oxygen saturation.

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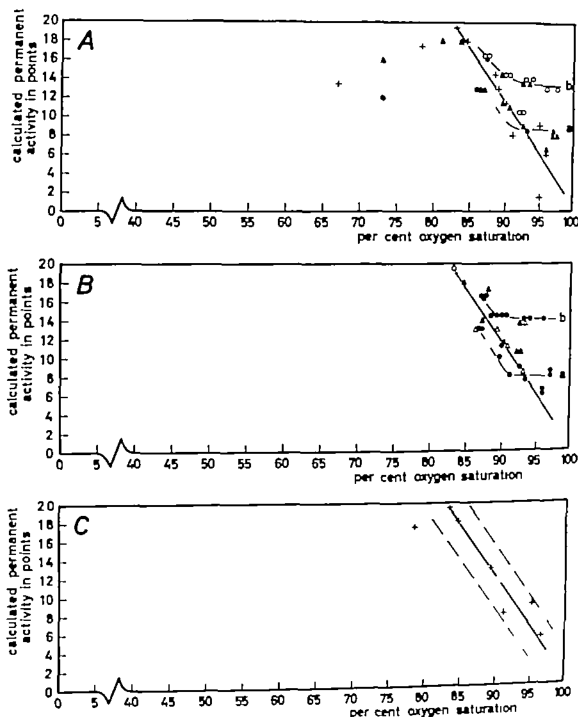


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B. Values as in 1 A. The symbols indicate alkalosis (△) hypercapnia (○) and other conditions (●)

C. Values from cases with cute asthma given separately

For further explanation: see text.

insufficiency they behaved differently. In one person definite permanent activity and "synchronism" was recorded after five minutes when the oxygen saturation was 85 per cent and arterial pCO was 49 mm Hg. He then became agitated, shuffled his feet unceasingly and interrupted the experiment after eight minutes as he felt the situation unbearable. Another person exposed to a similar increase in dead space and breathing against a greater external resistance accomplished experiments of up to 40 minutes duration when concentrated on the task of keeping relaxed and emptying the lungs after each inspiration either by forced expiration or by prolongation of the expiration period. In this subject no or weak permanent activity was recorded. "Synchronism" at the onset of inspiration was hardly seen at oxygen saturations from 62 to 93 per cent. The values for pCO varied from the normal range up to 54 mm Hg. As soon as this person concentrated less the expiration became ineffective and the experiment had to be interrupted.

These experiments demonstrate, that an increase in permanent activity can occur also in subjects with normal lungs and airways under conditions causing inefficient respiration associated with a state of anxiety. On the other hand severe breathing difficulties can be tolerated and permanent activity avoided if the muscular activity is kept under control.

Discussion

The calculated permanent activity is based on a crude estimation of the degree of permanent activity in the muscles. The value is not influenced by the marked activity sometimes recorded from one or two areas at the time when no or only low grade activity is obtained from other muscles. Such marked activity, mainly from m. sacrospinalis and m. trapezius, varied with the posture as may also part of the activity influencing the score, but if an influence is only local its effect on the total score is hardly significant. The calculated permanent activity is therefore assumed to be a crude measure for that part of the total permanent activity which is caused by generally acting mechanisms.

The findings in the patients with emphysema and asthma indicate that the muscular activity in general varies with the degree of arterial oxygen saturation. The finding of a higher activity in nervous than in non-nervous patients was not dependent on lack of oxygen and presumably not on alterations regarding pH or pCO_2. As an increased muscle tone is a common finding in neurotic patients without respiratory disorders (1, 4, 5, 6, 7) we think that the stronger calculated permanent activity in the nervous patients with emphysema and asthma is caused by central disturbances associated with the nervous state.

The different results obtained in two normal subjects indicate that respiratory insufficiency is tolerated better and permanent activity is reduced if the person concerned can ignore his sensation of stress and stays relaxed.

The finding of "synchronism" at the onset of inspiration in subjects with arterial hypoxia is similar to the results of studies on the activity of respiratory neurones in the bulb of decerebrate and decerebellat preparations. Under these experimental conditions the activity of expiratory neurones is suddenly replaced by activity of inspiratory neurones at the onset of inspiration (8). It seems therefore reasonable to assume that syn-

Table III Number of patients with emphysema and asthma and also "synchronism" in subgroups according to the oxygen saturation

Diagnosis	Oxygen saturation					
	< 92 %		92–94 %		> 94 %	
	Total examined	No. with "synchronism"	Total examined	No. with "synchronism"	Total examined	No. with synchronism
Acute asthma	6	6	0	0	3	0
Chronic asthma	8	8	4	2	2	0
Emphysema	11	11	3	1	5	0
All cases	25	25	7	3	10	0

two populations representing chronic respiratory disorder is independent of the origin of the values, being obtained either in wheezing asthmatics or in non wheezing patients with emphysema

The total series was analysed with regard to a possible correlation between pH and pCO_2 on the one hand and calculated permanent activity on the other No correlation was found

Factors examined with a view to their importance for the occurrence of "synchronism in patients with emphysema and asthma

An increased activity during inspiration was found in all patients, while expiratory activity was lacking in 50 per cent and was negligible in 17 per cent. An evaluation of the times for the appearance of inspiratory activity and the disappearance of expiratory activity in the muscles examined was possible in 42 cases.

Synchronism always occurred when the oxygen saturation was below 92 per cent and never when above 94 per cent (table III) No dependence on pCO_2 or pH was found

Several patients suffering from emphysema with or without asthma had a strong feeling of air hunger In order to estimate the influence of dyspnea due to other causes than emphysema and asthma a few patients with severe dyspnea and two normal subjects with experimentally induced respiratory insufficiency were studied.

Studies on dyspneic patients without emphysema and asthma

The values for calculated permanent activity and the results of blood analyses are given in table II In the patients with pulmonary fibrosis and lung cancer the calculated permanent activity was 6 and 9 5 points at oxygen saturations of 87 and 90 per cent respectively In the two patients with cardiac disease a similar permanent activity was found at oxygen saturations of 94 and 96 per cent. Thus, increases in the permanent activity can be found in patients with respiratory insufficiency caused by diseases other than emphysema and asthma.

Studies on normal subjects with experimentally induced respiratory insufficiency

Two trained subjects were used When standing at ease and breathing normally no permanent activity was recorded from their superficial muscles. Under the experimental conditions causing respiratory

insufficiency they behaved differently. In one person definite permanent activity and "synchronism" was recorded after five minutes when the oxygen saturation was 83 per cent and arterial pCO_2 was 49 mm Hg. He then became agitated, shuffled his feet unceasingly and interrupted the experiment after eight minutes as he felt the situation unbearable. Another person exposed to a similar increase in dead space and breathing against greater external resistance accomplished experiments of up to 40 minutes duration when concentrated on the task of keeping relaxed and emptying the lungs after each inspiration either by forced expiration or by prolongation of the expiration period. In this subject no or weak permanent activity was recorded. "Synchronism" at the onset of inspiration was hardly seen at oxygen saturations from 62 to 93 per cent. The values for pCO_2 varied from the normal range up to 54 mm Hg. As soon as this person concentrated less the expiration became ineffective and the experiment had to be interrupted.

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chronism occurs whenever automatic regulation from the bulb undisturbed by impulses from higher centres, dominates the activity of the motor units.

Summary

The study included 72 subjects with varying degrees of respiratory dysfunction caused by emphysema asthma, and other disturbances. The electromyographic activity of the diaphragm and a number of muscles of the neck and trunk was recorded just before determinations of arterial oxygen saturation pH and pCO_2.

A crude estimation of the degree of permanent activity in the superficial muscles was used. By adding scores given for the activity of each muscle an expression was obtained called calculated permanent activity

Calculated permanent activity varied with the mental state being definitely stronger in nervous than in non nervous subjects with chronic emphysema with or without asthma.

Calculated permanent activity varied with the arterial oxygen saturation Values obtained during attacks of acute intermittent asthma showed an almost linear increase with decreasing oxygen saturation to 85 per cent. For values obtained in non nervous and nervous patients with chronic emphysema with or without asthma an increase with decreasing oxygen saturation was indicated for values of 92—90 per cent down to about 85 per cent.

Results obtained in trained normal subjects demonstrate that an increase in permanent activity caused by breathing with an increased dead space against an external resistance can be avoided, if the person concentrates on the task to keep relaxed and prolongs his expiration.

"*Synchronism* the appearance of inspiratory activity and disappearance of expiratory activity in the accessory respiratory muscles at the moment for the onset of diaphragmatic activity occurred during non-volitional breathing when oxygen saturation was below 92—94 per cent

Acknowledgements

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Persistent Hyperchromic Erythrocytes in Pernicious Anaemia in Remission

By

S. Eriksson, P. G. Reizenstein and A. Sollberger

It has been suggested that the remission obtained in pernicious anaemia (p. a.) is not complete (1, 6, 7, 8, 13) but most reviewers reject these suggestions (2, 4, 12). The evidence that can be obtained from published data is controversial (3, 5, 9).

It is for these reasons, that the present study was performed. A simple criterion of "the degree of remission" the mean cellular haemoglobin (MCH) was studied in 62 patients with pernicious anaemia with an average duration of 9 years. Statistically the MCH remained high.

Material

Sixty-two patients with diagnosis of p. a. were studied, twenty-one men and fourty-one women. The diagnostic criteria are shown in table I. The diagnosis had been made on an average 9 (1—21) years prior to the present study and the patients had been coming to the Serafimerlasarettet Clinic for regular visits since this time, usually each month.

Several patients were treated with monthly injections of liver extract up to 3 years preceding the present study since then all received monthly injections of on an average, 78 (25—500) μg crystalline B_{12}. Thirty patients were given 50 μg/month, 20 were given 100 μg/month and only 12 had more.

Two different control groups were used. The first consisted of sixty-one control patients visiting the same clinic, who were selected at random. The charts alphabetically adjacent to those of the pernicious anaemia patients were selected, unless this adjacent chart carried diagnosis or evidence of anaemia, sideropenia, or bleeding. Twenty one of the control patients were men, and fourty were women.

The second control group consisted of 39 healthy volunteers (students, doctors, nurses) 10 men and 29 women.

Methods

Haemoglobin concentrations were measured with a Ljungberg colorimeter taking 16g/100 ml as 100 and red blood cell counts (RBC) were performed in the usual manner using Buerker counting chamber. All determinations, both in p. a.-patients, control patients, and volunteer controls, were performed by the same person, using the same instruments. In the p. a.-patients, the average

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chronism occurs whenever automatic regulation from the bulb undisturbed by impulses from higher centres, dominates the activity of the motor units.

Summary

The study included 72 subjects with varying degrees of respiratory dysfunction caused by emphysema asthma and other disturbances. The electromyographic activity of the diaphragm and a number of muscles of the neck and trunk was recorded just before determinations of arterial oxygen saturation pH and pCO_2.

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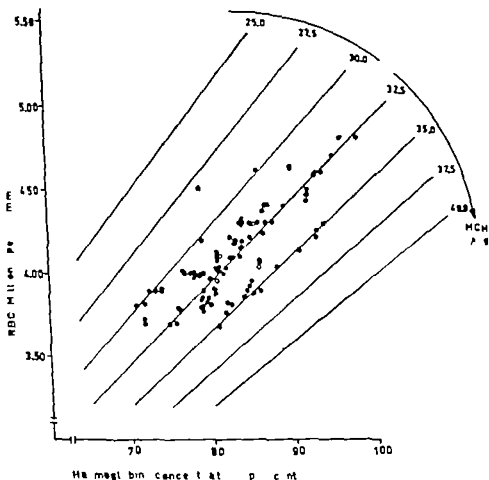


Fig. 1. Haemoglobin concentrations and RBCs in p. a.-patients in remission (○) and in controls (●). The means of several determinations in each person is shown. The slightly diverging lines represent the MCH-values, and 32.5 μμg seems to be rough limit value.

Regression coefficients: controls 0.0423 ± 0.00174, p. a. 0.0306 ± 0.00173; correlation coefficients controls 0.94, p. a. 0.92.

Consequently the mean cellular haemoglobins (MCH) are significantly higher in p. a. than in all control groups.

The mean age in the p. a. patients was 67 years, but it was only 51 years in the controls. The higher age might have contributed to the lower RBC in the p. a.-group. However, no significant correlation between RBC and age was found (fig. 2). Moreover, a statistically significant difference of MCH between controls and p. a.-patients remained even when the younger controls were excluded so that a control group with an age comparable to that in the p. a.-group was obtained (table IV).

As in most retrospective studies, the diagnosis of p. a. was uncertain in some cases. However, if healthy persons had been included in the p. a.-group this

Table I Diagnostic criteria. In the last 4 groups some symptoms are absent because of incomplete investigation

	No. of patients	
	Male	Female
Megaloblastosis + achlorhydria + anaemia reacting to B_{12}	4	9
Megaloblastosis + anaemia reacting to B_1	3	9
Megaloblastosis without anaemia	3	6
Achlorhydria + anaemia reacting to B_1	2	1
Anaemia reacting to B_1	9	16

10 of these cases had been treated by the referring physician.

Table II Errors of the methods

	Controls		P.A.	
	No. of observations	Error	No. of observations	Error
RBC (mill./mm³)	36	0.17	40	0.11
Haemoglobin (g/100 ml)	36	0.61	40	0.43
MCH ($\mu\mu g$/erythrocyte)	—	—	90	1.43
			91	1.90

The S.D. $\times 2^{-\frac{1}{2}}$ of the difference between 18 or 20 individual paired observations with time difference of about 1 month.
The S.D. of many observations made over 6.5 and 6.8 years, respectively in 2 single cases.

haemoglobin concentrations and RBC:s for each patient were calculated from the values obtained during the 5 last visits, i.e. over a period of about 5 months. In the control patients, an average for each patient was calculated from as many determinations as could be found, between 1 and 5. In each of the volunteer controls, 1 determination was done.

Table III Mean haemoglobin and erythrocyte values for controls and cases of P.A. Each group of values signifies. Mean ± S.E., S.D. No. of cases in brackets

Material	Men	Women	Both sexes
	Haemoglobin, (100% = 16 g/100 ml)		
Volunteer controls	92.7±1.9 6.2 (10)	79.2±1.0 5.3 (29)	
Control patients	85.1±1.3 5.9 (21)	80.9±0.9 5.5 (40)	
All controls	87.5±1.2 6.9 (31)	80.2±0.7 5.5 (69)	82.5±0.7 6.8 (100)
P.A.	87.6±1.2 5.5 (21)	82.5±0.8 4.9 (41)	84.1±0.7 5.7 (62)
	RBC, mill./mm³		
Volunteer controls	4.64±0.10 0.32 (10)	4.02±0.05 0.24 (29)	
Control patients	4.30±0.06 0.27 (21)	4.10±0.04 0.23 (40)	
All controls	4.41±0.06 0.33 (31)	4.06±0.03 0.24 (69)	4.17±0.03 0.31 (100)
P.A.	4.06±0.04 0.16 (21)	3.88±0.03 0.18 (41)	3.94±0.02 0.19 (62)

Since repeated determinations were performed in single subjects the error of the methods, including the effect of variation in time could be calculated (table II).

Results and discussion

The haemoglobin and RBC-values are shown in table III and fig. 1 and 3.

The haemoglobin values in p.a. are numerically higher than those in all control groups except the (young) male volunteers (table III).

Nevertheless the RBC:s are always lower in p.a. (table III, fig. 1 and 3).

and did thus not differ from that in the other p. a.-patients.

Conceivably some of the p. a.-patients might have been in partial relapse and this could explain their high MCH. However one would expect such patients to have a sub-normal haemoglobin value and it is seen in fig 1 that even the p. a.-patients with high haemoglobin values had low RBC counts and thus high MCHs. Moreover a statistically significant difference between the MCH in controls and in p. a. patients was found even when only those p a.-patients were included whose haemoglobin was over 11.5 g/100 ml, and where a partial relapse was therefore unlikely (table IV)

Since the control patients were not healthy a higher frequency of iron deficiency and thus a lower average MCH than in healthy persons cannot be excluded. It is not likely that iron deficiency would be more common in this control group than in the patients with p. a., for both the advanced age and the achlorhydria can cause iron deficiency in p. a In addition the MCH in the control patients was not lower than in the volunteer controls (table IV) Moreover iron deficiency would not explain the difference between the volunteer controls and the p. a.-patients (table I)

The present study does not contain sufficient information to explain the persistent macrocytosis in p a. in remission. Neither do previously published papers. Kirk (5) found that, although individual mean cellular volumes became normal, the mean cellular diameters remained high in p. a. after about 4 weeks of treatment with a total of 40 ml liver extract (about 1,500 μg hydroxocobalamin) Larsen (6 7) made similar findings after some 400 ml liver extract, 0.15 g folic acid, and 480 μg B_{12}.

Table II MCH in P A., in total controls, and in selected control groups. (Not even in control selected to be over 50 year of age is the MCH as high as in P A Only control patients over 57 and volunteers over 50 were included in the "old control"-group)

Material	MCH, $\mu\mu g$ Mean ± S. E.	No. of pat.	Average age
All control patients and volunteer controls	31.6 ± 0.09	100	51
Control patients	31.9 ± 0.10	61	54
Volunteer controls	31.7 ± 0.14	39	45
"Old" controls	31.5 ± 0.12	50	62
All P.A. patients	34.0 ± 0.12	62	67
P.A.-patients with haemoglobin concentration over 11.5 g 100 ml	34.3 ± 0.15	46	67

Table V MCH in P A. in remission treated with different amounts of B_{12} (8 patients were not included in this table because they had received varying amount of B_{12})

Monthly B_{12}-dose (μg month)	No. of cases	MCH Mean ± S. E.
≤ 50	28	34.00 ± 0.19
51 – 100	17	34.00 ± 0.26
> 100	9	34.60 ± 0.40

Philipson (9) found a remaining high MCH but his patients often had low serum-B_{12} levels. In contrast, Hallberg (3) whose patients were given 50 μg B_{12} every week or every second week, found an average MCH in 21 p. a.-patients which did not differ from the value found in controls.

The most probable reason for the low MCH found in the present series is that the patients were given too little B_{12}. An attempt was made to correlate the

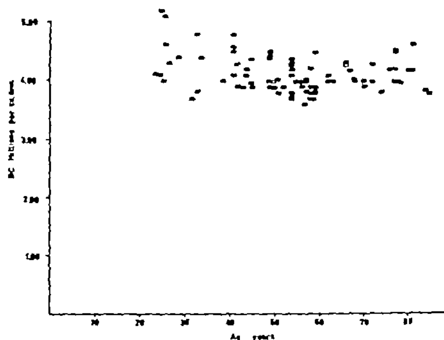


Fig. 2 Relationship of RBCs to age in all controls (men ■, women □)

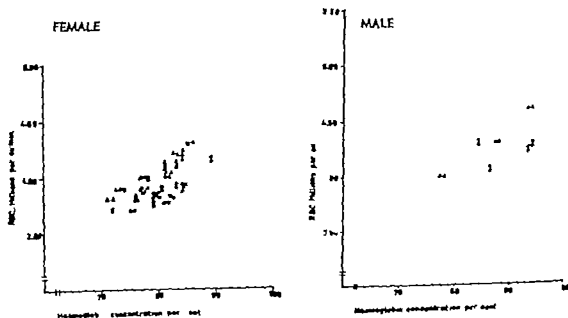


Fig 3. Haemoglobin concentrations and RBCs in femal p. a-patients (○) volunteer controls (●) and control patients (Δ) and the same male sub-groups. It is seen that the difference in MCH between p. a.-patients and controls is present in all sub-groups.

would hardly simulate a macrocytosis and the normal haemoglobin values after B_{12}-therapy make it unlikely that macro- ... from causes other than

B_{12}-deficiency were included The MCH in the 10 female patients in table I whose diagnoses had been made by the referring physician was $34\ 00 \pm 0.28$

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Frequency of "Abnormal" Serum Globulins (M-Components) in the Aged

By
J. HÄLLÉN

Serum electrophoresis will occasionally show an increase of a certain globulin, a so-called M-component (13), i.e. an atypical concentration gradient suggesting an increased production of a certain protein — possibly a number of proteins of largely the same mobility. Waldenström (21) has called this monoclonal hypergammaglobulinaemia. These M-components appear as typically situated narrow bands in the paper electrophoretic pattern. Whether they should be regarded as a manifestation of a globulin normally occurring in extremely low concentration or as a globulin not normally occurring is debatable, but as far as the present investigation is concerned this is irrelevant.

The occurrence of M-components directs one's thoughts above all to myeloma and Waldenström's macroglobulinaemia. Such components have, however, also been observed in various malignant diseases (1, 4, 6, 10, 12, 18) and in apparently healthy individuals (9, 11, 16, 17, 20). All of the abovementioned conditions occur mainly in elderly persons. It was therefore considered worthwhile to assess the frequency of M-components in apparently physically healthy aged persons.

Submitted for publication December 14, 1962.

Material

The material consisted of sera from all persons above 70 years of age living at the two homes for old people at Malmö, with the exception of those who were unwilling to co-operate or who were bed-ridden because of some acute disease. People are admitted to these homes on social grounds (poor homes, death of husband or wife, loneliness etc.) and not for medical reasons. Illness is, if anything, a contraindication, for these homes accept only persons able to take care of themselves (dress themselves, walk to the dining-rooms, etc.) at the time of admission. In order to secure a larger material, sera were also collected from 40 patients, likewise at least 70 years of age, at two female departments for senile dementia of a mental hospital (Malmö Östra Sjukhus, MÖS).

MCH to the dose of B_{12} (table V) but no obvious relationship could be found This may be because all doses used were too low (10 11) or because a single monthly injection simply does not permit of a sufficient amount of B_{12} to be retained (10 a) However the occurrence of irreversible damage during B_{12}-deficiency the possible existence of other deficiencies than B_{12}, or deficient conversion of cyanocobalamin to non-cyanoform (11 a) cannot be ruled out

Summary

Sixty two patients with pernicious annemia of 9 years duration were studied They were in remission received 78 µg B_{12} parenterally per month and their individual blood values were within normal limits Nevertheless the mean cellular haemoglobin of the group continued to differ significantly from that in a group of 61 control patients and 39 volunteer controls it remained high These patients, therefore were not in complete remission. The reason may be treatment with too little B_{12}

Acknowledgments

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Case 5. Female, aged 90 years. The woman had felt well until 1950. She was then admitted to the old people home because of fatigue loneliness and forgetfulness. On admission she was found to have slight anaemia (Hb 70), which responded well to iron therapy and the liver was felt 2 finger breadths below the right costal arch. That year she also had a basal cell cancer on the back, which responded favourably to roentgen treatment. I 1961—1962 she became increasingly senile. In January 1962 an M component was discovered in the serum. In the spring of 1962 she complained of symptoms reminiscent of cholecystitis. Palpation revealed lump suggestive of stone-filled gallbladder. In July 1962 the patient was examined because of the detection of a serum M-component she was moderately senile the gallbladder and the liver were palpable as before. Physical examination revealed no other abnormalities. Roentgen examination of the skull showed no signs of myeloma.

Case 6. Male, aged 80 years. The man had syphilis in 1905. From 1931 to 1944 he had spent several periods in hospital for treatment of his syphilis. When last examined in 1944 the serological examinations of the CSF no longer gave positive findings, but one serum test (Meinicke) was positive. During the following years he felt well. At examination in 1962 because of the discovery of an M-component he was found to be moderately senile, the pupils did not react to exposure to light, and the knee and ankle jerks could not be elicited. Physical examination revealed no other abnormalities. Serological tests for syphilis were negative. Roentgen examination of the skeleton showed no signs of myeloma.

Case 7. Male, aged 89 years. The man had felt well until the last few years during which he had suffered from breathlessness, which had been regarded as symptom of cardiosclerosis and treated with digitalis. He was sometimes disorientated. At physical examination because of the detection of an M-component in the serum he was found to be well orientated, the lips were cyanosed, the fingers slightly clubbed, the legs were somewhat oedematous and the respiratory sounds over the lungs were faint. Roentgen examination showed emphysema and changes suggestive of bronchiectasis; the skeleton showed no signs of myeloma.

Case 8. Male aged 91 years. He had always felt well. Since 1950 he had lived at the old people home. During the last year he had felt tired and weak. Physical examination because of the discovery of an M-component in the serum revealed nothing remarkable. Roentgen examination of the skull showed no signs of myeloma.

Case 9. Male aged 87 years. In 1958 he was admitted to hospital for very transient symptoms of a minor stroke. He has since lived at the old people's home where he has always felt well. Physical examination following the discovery of M-components in the serum revealed nothing remarkable. Roentgen examination of the skeleton showed no signs of myeloma.

Results

M-components were found in 9 persons — 6 men and 3 women. The mean age of this group was 84 years. One woman belonged to the MÖS-group.

It is clear from the case reports that 3 persons (cases 3 4 5) had or had had cancer. One (case 3) of them died of prostatic cancer a few months after discovery of the M-component in the serum. Further clinical investigation and laboratory studies in this case were therefore incomplete but necropsy was done. The second (case 4) had been operated upon for gastric adenocarcinoma 5 years previously. The tumour had by then produced symptoms for about 1 year. At the time of the present investigation he was symptom-free. The third (case 5) had received roentgen therapy for basal cell cancer which had responded well.

One person (case 1) was severely demented one (case 2) had sequelae

The material consisted of all together 294 persons — 135 men and 159 women. The mean age was 81 and was the same for both sexes. The mean age of the group from MÖS (80 years) was practically the same as for the entire material.

Methods

Paper electrophoresis was done by the method of Laurell et al. (8). In all cases in which the serum was found to contain an M-component the examination of the patients was extended to include inquiry into the *history*, general physical examination, roentgen examination of the skeleton including skull, spine, chest and pelvis — in some cases only the skull — determination of the erythrocyte sedimentation rate (E.S.R.), haemoglobin (Hb), number of red and white blood cells, thrombocytes and differential count as well as sternal puncture. Immunoelectrophoresis, starch gel electrophoresis and paper electrophoresis of the urine were done at the Department of Clinical Chemistry, Malmö General Hospital by methods recently described by Bachmann and Laurell (2).

Case reports

Case 1. Female, aged 83 years. The woman had felt well until 1955, when she became increasingly confused and irritable and was therefore admitted to MÖS, where she has since been cared for. She has had no physical illness during her stay there. On examination in 1962, after discovery of the M-component in the serum, the patient was severely demented. She was very thin. The liver and the spleen were not palpable and no enlarged lymph nodes could be felt. Roentgen examination of the skeleton was less successful but definitely excluded changes characteristic of myeloma in the skull.

Case 2. Female, aged 70 years. The woman had felt well until 1937, when extirpation of a myoma was followed in the night by total hemiparesis with aphasia, which became fully developed within a few hours. Recovery was only partial and she has since been unable to work. In 1959 she was admitted to the old people's home. No change in her condition had been noted during the last 10 years. Physical examination in 1962 because of the discovery of an M-component in the serum revealed nothing of interest except the neurological symptoms. Roentgen examination of the skull showed no signs of myeloma.

Case 3. Male, aged 83 years. The man had felt well until 1956, after which he had spells of pain resembling sciatica. He was admitted to the old people's home in 1961. At that time he had leg oedema of supposed cardiosclerotic origin. In the autumn of 1961 he developed anaemia. In January 1962 the serum was found to include an M-component. From the same time he had begun to have backpain. At later examination palpation suggested prostatic cancer, and roentgen examination showed widespread skeletal changes supporting this diagnosis. The pain increased in intensity, the patient deteriorated and died in June 1962. Necropsy showed general arteriosclerosis with signs of cardiac failure, prostatic cancer with skeletal metastases and no signs of myeloma.

Case 4. Male, aged 79 years. The man had felt well until 1955, when he had a pertrochanteric femoral fracture which healed satisfactorily. In 1956 he was operated upon for inguinal hernia, and then sideropenic anaemia was discovered as well as the presence of blood in the stools. Roentgen examination showed a gastric polyp, the size of a hen's egg, but the patient was considered too poor a risk for operation; he was given blood transfusions and sent home. In 1957 he was admitted to the medical department because of cardiac failure and angina pectoris, which were believed to be due to anaemia. Electrophoresis showed the albumin to be slightly decreased and the α_1 and α_2-globulins to be increased, but no M-component could be detected. On that occasion the polyp was excised and proved to be an adenocarcinoma. He afterwards felt well except for mild symptoms of cardiac failure. Physical examination in 1962 because of the discovery of an M-component in the serum revealed nothing remarkable. Roentgen examination of the skeleton showed moderate osteoporosis of the spine but no bone destruction characteristic of myeloma.

Paper electrophoresis — serum (g/100 ml)							M-component			
							In serum; paper electrophoresis (g/100 ml)	Mobility on paper electrophoresis, serum	Immunological type	In urine; paper electrophoresis
Total protein	Alb.	α	α	β	β	γ				
7.5	4.4	0.34	0.51	0.40	0.34	1.10	0.9	[illegible] $-\gamma$	γ[illegible]	0
8.4	4.1	0.38	1.31	1.06	0.46	1.31	~1.2	$\alpha-\beta$	γ (atypical)	+
4.8	3.0	0.28	0.54	0.30	0.52	0.24	~0.3	$\beta-$[illegible]	[illegible]	+
7.5	4.8	0.29	0.34	0.45	0.27	1.10	0.4	$\gamma-\gamma$	γ1M	0
7.9	4.6	0.34	0.49	0.41	0.26	0.80	1.0	$\gamma-\gamma$	γ[illegible]	0
8.6	2	0.27	0.71	0.39	0.30	0.70	1.0	[illegible] $-\gamma$	γ[illegible]	0
8.1	4.9	0.34	0.48	0.49	0.32	0.90	0.7	α	γ1A	0
7.5	4.5	0.36	0.62	0.43	0.31	0.90	0.4	γ	[illegible]	0
8.3	5.0	0.32	0.51	0.48	0.32	0.80	0.3+0.5	$\gamma-\gamma$	γ[illegible]	0
6.4–7.8	4.2–5.3	0.23–0.38	0.34–0.59	0.39–0.62	0.24–0.42	0.65–1.10				

blood cells was 3 100/mm³ and in the other cases it varied between 4 100 and 7,900/mm³. The differential counts were normal. In 7 cases the thrombocytes were counted and were found to be less than 150,000/mm³ in 3. The above-mentioned counts were determined only once.

The E. S. R. was clearly elevated in 2 cases. In case 7 in which it was 34 mm/1 hour and in which the emphysema and possibly bronchiectasis suggested chronic bronchitis, the plasma fibrinogen was 0.43 mg/100 ml (normal 0.22—0.34 mg/100 ml) while the "normal" gammaglobulin fraction was of normal, and the M-component of moderate concentration (0.7 g/100 ml). In case 6 the E. S. R. was 60 mm/1 hour and there was no reason to suppose active infection the fibrinogen was somewhat high (0.46 mg/100 ml) the normal gamma fraction 0.7 and the band 1.0 g/100 ml.

Hypoalbuminaemia was found only in the man with prostatic carcinoma. The α_1-globulin was normal in all. α was moderately increased (0.71 g/100 ml) in the man with emphysema and an E. S. R. of 34 mm/1 hour. The "normal" gammaglobulin fraction was slightly increased in one case (No. 2) and decreased in one (No. 3). In case 2 the serum contained an atypical micro-molecular M-component, which could not be separated from the normal α_2-β_1 and β_1-fractions and was thus only approximately estimated. Case 9 showed two narrow bands in the γ_1-region. The abnormal protein in case 3 could not be separated from the β_2-fraction and

Table I Data on 9 cases with M-components

Case	Sex	Age	Hb (g/100 ml)	WBC (/mm³)	Thrombocytes (1000/mm)	Bone marrow	ESR
1	♀	83	13.2	4,800	117	Plasma cells 72/1 000	23
2	♂	70	12.5	5 400	192	Plasma cells 48/1,000	16
3	♂	83	11.5	5,200	—	Prostatic cancer	25
4	♂	79	15.5	7,900	220	Normal	11
5	♀	90	12.0	4,500	24	Normal	29
6	♂	80	12.8	4 100	156	Normal	60
7	♂	89	14.9	3,100	138	Normal	34
8	♂	91	14.2	5,000	—	Normal	16
9	♂	87	12.8	6,200	96	Normal	26

Normal range for healthy blood donors

Normal globulin + M-component.

after a cerebral lesion which was regarded as a postoperative complication in an otherwise healthy person one (case 6) had symptoms suggestive of tabes dorsalis one (case 7) had emphysema, and clubbed fingers In one woman (case 5) the liver was slightly enlarged but in none of the others was the liver or spleen palpable and none had enlargement of the lymph nodes nor were there any signs of haemorrhagic diathesis. These were the only physical findings of interest in the above mentioned 9 individuals.

In 5 cases the investigation included roentgen examination of the skeleton but in one the result was technically less successful except concerning the skull. In 3 cases (nos. 2 5 8) only lateral views were taken of the skull No bone destructions characteristic of myeloma were seen

Histological examination of bone marrow in one case (No 3) showed widespread metastases from the prostatic cancer but no signs of increased plasma cell proliferation Of the remaining 8 cases cytological examination of sternal punctate showed more than 30 plasma cells per 1 000 nucleated cells in two (No. 1 and 2 with 72 and 48/1 000) in both of which the plasma cells were of normal appearance In the other 6 examination revealed nothing remarkable

Most of the following laboratory data are grouped together in table I

Only the man with prostatic cancer (case 3) had an obvious anaemia In one case (No. 7) the number of white

Paper electrophoresis — serum (g/100 ml)							M-component			
Total protein	Alb.	α	α	β	β	γ	In serum; paper electrophoresis (g/100 ml)	Mobility on paper electrophoresis, serum	Immunological type	In urine; paper electrophoresis
7.9	4.4	0.34	0.51	0.40	0.34	1.10	0.9	γ – ,	γ–	0
8.4	4.1	0.38	1.11	1.06	0.46	1.31	~1.2	α – β	? (atypical)	+
4.8	3.8	0.28	0.56	0.50	0.52	0.24	~0.3	β – γ	γ1A	+
7.3	4.8	0.29	0.54	0.43	0.27	1.10	0.4	γ – γ	γ1M	0
7.9	4.6	0.34	0.49	0.41	0.26	0.80	1.0	γ – γ	γ–	0
8.6	5.2	0.27	0.71	0.59	0.50	0.70	1.0	γ – γ	γ–	0
8.1	4.9	0.34	0.48	0.49	0.32	0.90	0.7	α_2	γ1A	0
7.5	4.5	0.36	0.62	0.43	0.31	0.90	0.4	γ	γ–	0
8.3	5.0	0.32	0.51	0.48	0.32	0.80	0.3+0.5	γ – γ	γ–	0
6.4–7.8	4.2–5.3	0.23–0.38	0.34–0.59	0.39–0.62	0.24–0.42	0.63–1.10				

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was thus only approximately estimated. The concentrations of the M-components could thus be determined in 7 cases, in which it varied between 0.4—1.2 g/100 ml The rate of migration varied considerably from γ_2 to α_2

Immunoelectrophoresis showed that the M-components were of immunological γ-type (γ_{ss}) in 5 cases including the one (No 9) with two bands. One (case 2) of the women with an increased number of plasma cells in the bone marrow had a micromolecular component (γ_{ss}) cases 3 and 7 a γ_{1A} and case 4 a γ_{1M}-component in the serum. The γ_{1M}-globulin like the γ_{ss}-component in case 5 did not penetrate the starch gel. Paper electrophoresis of the urine showed leakage of microcomponents in cases 2 and 3

Discussion

Broadly speaking the 294 persons studied may be regarded as a random selection of aged persons. None of those living in the old people's homes had been admitted there for medical but for social reasons. People in the homes belong however mainly to the lower social classes and may represent a selection of persons with rather pronounced symptoms of ageing This applies, above all, to the 40 patients at MÖS The state of health of the 9 with M-components in the serum leaves the impression that they represented a diseased group But 6 of them were healthy on admission to the home. Of the remaining 3 one had sequelae after a hemiplegia but otherwise felt well, one was somewhat senile (possibly accentuated by previous cerebrospinal syphilis) and one — from MÖS — had senile dementia. Nothing suggests that M-components are so common in these conditions, that the inclusion of these 3 persons and those from MÖS should bias the material and result in a spuriously high incidence of M components

It is clear from the results that the M-components in persons above 70 years of age were more common than expected A search of the literature failed to reveal any *systematic investigation of* the frequency of M-components in the aged In the series of Heremans et al. (6) which covered some 30 000 sera, the frequency was only about 1 % in spite of the fact that here cases with abnormal proteins were overrepresented, for the sera had been collected from hospital patients and had been further supplemented with sera with M-components

That one third of the persons with M components in the present small group had cancer may be ascribed to chance. It can, however be explained by the high average age of the series and by the fact (4 7 12 15) that M-components sometimes occur also in malignant diseases without relation to the reticuloendothelial system The most illustrative of this type was the one (case 3) with widespread skeletal metastases from prostatic cancer a faint immunological γ-band and loss of an M-component in the urine. The occurrence of Bence Jones proteins in the urine in patients with skeletal metastases has been described in a few cases, *mainly in less* recent papers (14) and these proteins should be regarded *as some of the* abnormal globulins sometimes demonstrable *in the urine*. The man (No 4) who was operated upon for gastric adenocarcinoma in 1957 and whose serum then showed *non specific electro-*

phoretic changes but no M-component is of particular interest. He has since felt well, and when last seen in 1962 he was in a good general condition, the E. S. R. was normal and the electrophoretic pattern was normal apart from a narrow band of γ_{1M}-type. The co-occurrence of macroglobulins and cancer has long been known (15) and there might therefore be reason to suspect some connection between the operated gastric tumour and the "abnormal" protein in this case. Is the presence of an M-component, for example a sign that the patient still harbours a malignant tumour? It is still too early to answer this question.

In none of the 9 cases could a diagnosis of multiple myeloma be established. Neither could it be excluded except in case 3, where post mortem only showed widespread skeletal metastases. It is difficult to say anything about the significance of the moderately and slightly increased number of plasma cells, respectively in the bone marrow in cases 1 and 2. Such increases have been observed in conditions other than myeloma (3, 19) (in particular in hypergammaglobulinaemia, chronic infections, LED, liver cirrhosis, etc.) but no evidence of such diseases could be demonstrated in these 2 cases and the "normal" γ-fraction was slightly elevated only in case 2. None of the 9 had grave anaemia and none showed skeletal evidence of myeloma. The serum in case 2 contained M-component of 7 S-type, and it should be observed that in the series of Heremans et al. (6) 6/7 of the patients with this type of globulin had myeloma. In view of the relatively low concentrations of M-components in these 9 cases, it is tempting to suspect "essential" monoclonal hypergammaglobulinaemia (21).

None in the group showed the typical clinical picture of Waldenström's macroglobulinaemia. In case 4 the band was of γ_{1M} type but was only faint.

One man had a low "normal" γ-fraction, but neither he nor any of the others had a history suggesting reduced resistance to infection, which is known to occur in myeloma as well as in other conditions with narrow bands (5).

All immunological types of γ-globulin were represented in the group described. But the group is small and the patients have not been followed up long enough to permit of any conclusions regarding the clinical significance of the M-components.

Summary

Of 294 largely physically healthy persons above 70 years of age 9 were found to have serum proteins of M-component type. One was later found to have prostatic cancer and skeletal metastases. In none of these 9 persons could the diagnosis of myeloma or macroglobulinaemia Waldenström be established.

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Nitrogen, Lipid, Glycogen and Deoxyribonucleic Acid Content of Human Liver

The Effect of Brief Starvation and Intravenous Administration of Glucose

By

A. Martinsson, H. Sunzel and B. Hood

The conjugation of cholic acid measured in vitro in human liver tissue has been studied after a short period of starvation as compared with administration of glucose in large doses (17). The question arose how these factors would influence the protein and lipid levels.

Earlier and present studies on the relationships between serum and liver lipid values (9) also made it important to know how liver lipids are influenced by variations in the nutritional state before biopsy.

The results in animals on the effect of starvation or diet on the lipid content of the liver have not shown consistent results (10, 12) and seem to vary between different species. That starvation produces a decrease of the protein content of the liver has been established by a number of experiments in animals. We have not been able to find similar studies in man.

There is a widely accepted opinion that administration of glucose increases the protein content of the liver due to its protein-sparing effect. Direct evidence of this is lacking, the assumption being based upon the wellknown fact that administration of glucose decreases the nitrogen output in the urine.

The purpose of this study was to investigate the effect of short starvation as well as administration of glucose in large doses on the content of nitrogen, lipids, glycogen and deoxyribonucleic acid (D.N.A.) in liver biopsies taken from patients in connection with operation for peptic ulcer.

Clinical material and methods

The clinical material consists of 26 patients with peptic ulcer either of the stomach or the duodenum. The patients were operated with partial gastrectomy in combination narcosis as described in another work (17).

Preparation of patients

On the day before the operation, 13 patients were given the ordinary hospital diet and 13 patients starved from 8.00 a.m. Four

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Table I Total lipid of liver tissue

	Methanol-chloroform (g/100 g dried liver)	Ethanol-ether (g/100 g dried liver)
1	23.5	24.3
2	12.5	11.4
3	12.3	11.8
4	26.5	23.8
5	14.4	13.3
6	45.8	45.9
7	11.6	10.5

hundred g of glucose were given intravenously as a 20 % solution to 7 of the patients on ordinary diet and to 6 of those, who starved, at a rate of 0.4 g/kg body weight/hour half before luncheon and half in the afternoon.

Biopsy

Immediately after the peritoneal incision, a biopsy of the liver was taken from the margin of the left lobe by incision going to a depth of close to 2 cm. This did not lead to any complication in any of the cases. The tissue was weighed and homogenized in distilled water in a Potter Elvehjem apparatus.

The homogenate was stored at —16 C until further preparation could be started. It was then freeze-dried and stored for 7 days in a desiccator over pentoxide of phosphorus.

D.N.A. was determined by the method described by Lovtrup and Roos (11).

Nitrogen was determined by the method described by Kjeldahl. The error of a single determination was 3.5 %.

Glycogen was determined by the method described by van der Vies (19) as modified by Edlund, Isaksson and Sunzel (7). The error of a single determination was 1.6 %.

The freeze-dried homogenate was extracted in 30 ml methanol-chloroform, 1 1 v/v per 0.1 g tissue in the cold overnight. After partition dialysis by the method described by Folch et al. (8) chloroform was added to 50 ml. From this extract, aliquots were taken for analysis of the lipids. Lipid phosphorus was determined by the method of Svanborg and Svennerholm (18) and converted to phospholipids by multiplying by 25. The error of a single determination was 2.3 %.

Cholesterol was determined after saponification according to the Sperry and Webb modification (16) of the Schoenheimer and Sperry method (15). The error of a single determination was 4.1 %.

The triglycerides were determined by the method described by Carlson and Wadström (5) as revised by Carlson (6). The triglyceride was calculated as tripalmitine. The error of a single determination was 4.1 %.

Control of the extraction procedure

Seven samples were used for a check on the above extraction procedure with a Soxhlet extraction for 2 hours with ethanol-ether 3/1 v/v.

The ethanol-ether extracts were evaporated in vacuo dissolved in petroleum-ether transferred to a receptacle, evaporated by mild warming and dried in a desiccator before weighing. The results appear in table I. The methanol-chloroform extraction seems as efficient as the Soxhlet extraction with ethanol-ether.

The extraction was also checked by adding tripalmitine in substance to dried liver. The recovery was between 95—105 %.

Statistical methods. Standard errors of methods were calculated directly from consecutive duplicate determinations and are expressed as coefficient of variation, i.e. standard deviation as a percentage of the mean.

Relationships between variables were analysed with regression analysis of variance. Equations of regression lines and correlation coefficients were given. P values less than 0.05 were considered to indicate significance.

Results

The results of the analyses are given in table II.

Liver glycogen content per 100 g wet liver was lowest in the starved group (average 1.35 g) and highest in the group given ordinary diet + glucose (average 7.88 g). In the group starved and then given glucose the glycogen was of the same magnitude as in the group on ordinary hospital diet only (average 3.86 and 3.31 g respectively).

Table II. Dry weight, glycogen, phospholipid, cholesterol, triglyceride and DNA content of human liver. Effect of short starvation and administration of glucose

Patient	Experimental group	Dry weight (g/100 g wet weight)	DNA	Nitrogen	Phospholipid	Cholesterol	Triglyceride	Glycogen/100 g wet liver
			g/100 g dried liver					
S 30	Ordinary hospital diet	29	–	9.5	8.81	0.80	–	4.86
S 31		31	–	7.0	6.17	0.55	–	4.75
S 41		33	1.23	8.2	8.23	0.83	0.27	2.40
S 47		30	1.63	8.9	9.55	0.80	0.12	2.64
S 148		33	1.50	8.6	7.09	0.83	0.54	2.34
S 172		30	1.03	11.3	9.58	0.80	0.38	2.85
	Mean	31	1.30	8.9	8.24	0.77	0.33	3.31
	Range	29–33	1.03–1.63	7.0–11.3	6.17–9.58	0.55–0.83	0.12–0.54	2.34–4.86
S 26	Ordinary hospital diet + 400 g glucose I.	37	–	7.2	6.58	0.77	0.24	5.11
S 33		36	–	7.2	6.59	0.54	0.05	6.59
S 63		33	1.22	7.2	8.06	0.69	0.12	7.30
S 67		32	1.40	7.8	7.33	0.61	0.11	7.80
S 73		32	1.27	7.2	6.86	0.70	0.16	9.99
S 75		32	1.43	7.9	8.07	0.65	0.14	7.45
S 78		35	–	7.2	7.36	0.73	0.19	10.94
	Mean	34	1.33	7.4	7.24	0.67	0.14	7.88
	Range	32–37	1.22–1.40	7.2–7.9	6.58–8.07	0.54–0.77	0.05–0.24	5.11–10.94
S 44	Starvation for 24 hours	34	0.98	9.3	7.16	0.84	0.14	1.45
S 59		35	–	9.2	8.35	0.81	0.07	1.77
S 35		33	1.51	9.2	9.63	0.80	0.20	1.77
S 66		28	–	10.2	9.74	0.76	0.21	0.56
S 78		28	1.55	9.8	9.82	0.71	0.17	0.38
S 91		33	1.56	9.5	9.37	0.83	0.66	1.22
S 100		31	–	8.8	10.07	0.69	0.12	2.30
	Mean	32	1.30	9.4	9.33	0.77	0.22	1.35
	Range	28–35	0.98–1.51	8.8–10.0	7.16–10.07	0.69–0.84	0.07–0.66	0.38–2.30
S 120	Starvation for 24 hours + 400 g glucose I.	29	1.22	9.7	7.02	0.68	0.51	4.04
S 121		29	1.00	9.5	9.04	0.72	0.12	4.44
S 122		30	–	9.9	9.39	0.78	1.36	1.99
S 123		32	–	8.7	7.84	1.07	0.14	3.39
S 125		32	1.28	8.8	9.23	0.76	2.81	2.41
S 128		33	1.14	7.0	7.42	0.77	0.32	6.59
	Mean	31	1.16	8.9	8.32	0.80	0.91	3.86
	Range	29–33	1.00–1.28	7.0–9.9	7.02–9.39	0.38–1.07	0.12–2.81	1.99–6.59

Table I Total lipid of liver tissue

	Methanol-chloroform (g/100 g dried liver)	Ethanol-ether (g/100 g dried liver)
1	23.5	24.3
2	12.5	11 4
3	12.3	11.8
4	26.5	23.8
5	14 4	13.3
6	45.8	45.9
7	11.6	10.5

hundred g of glucose were given intravenously as a 20 % solution to 7 of the patients on ordinary diet and to 6 of those, who starved, at a rate of 0 4 g/kg body weight/hour half before luncheon and half in the afternoon.

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Immediately after the peritoneal incision, a biopsy of the liver was taken from the margin of the left lobe by incision going to a depth of close to 2 cm. This did not lead to any complication in any of the cases. The tissue was weighed and homogenized in distilled water in a Potter Elvehjem apparatus.

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Glycogen was determined by the method described by van der Vies (19) as modified by Edlund, Isaksson and Sunzel (7) The error of a single determination was 1 6 %

The freeze-dried homogenate was extracted in 30 ml methanol-chloroform, 1 1 v/v per 0 1 g tissue in the cold overnight. After partition dialysis by the method described by Folch et al. (8) chloroform was added to 50 ml. From this extract, aliquots were taken for analysis of the lipids. Lipid phosphorus was determined by the method of Svanborg and Svennerholm (18) and converted to phospholipids by multiplying by 25 The error of a single determination was 2.3 %

Cholesterol was determined after saponification according to the Sperry and Webb modification (16) of the Schoenheimer and Sperry method (15) The error of a single determination was 4 1 %

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Control of the extraction procedure

Seven samples were used for a check on the above extraction procedure with a Soxhlet extraction for 2 hours with ethanol-ether 3/1 v/v

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The results of the analyses are given in table II

Liver glycogen content per 100 g wet liver was lowest in the starved group (average 1 35 g) and highest in the group given ordinary diet + glucose (average 7 88 g) In the group starved and then given glucose the glycogen was of the same magnitude as in the group on ordinary hospital diet only (average 3 86 and 3.31 g respectively)

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			g/100 g dried liver					
S 30	Ordinary hospital diet	29	–	9.5	8.81	0.80		4.87
S 31		31	–	7.0	6.17	0.33	–	4.75
S 41		33	[illegible]	8.2	8.23	0.85	[illegible]	2.40
S 43		30	1.63	8.9	9.55	0.80	0.12	2.64
S 145		33	1.30	8.6	7.07	0.81	[illegible]	2.34
S 172		30	1.03	11.5	9.58	0.80	0.58	2.85
	Mean	31	1.30	8.9	8.24	0.77	0.33	3.31
	Range	29–33	1.03–1.63	7.0–11.5	6.17–9.58	0.33–0.85	0.12–0.54	2.34–4.87
S 5[illegible]	Ordinary hospital diet + 400 g glucose L	37		[illegible]	6.38	0.77	[illegible]	5.11
S 55		36		7.2	6.59	0.54	0.05	6.59
S 63		33	1.22	7.2	8.0[illegible]	0.69	0.12	7.30
S 67		32	1.40	7.8	7.35	0.61	0.11	7.80
S 73		32	1.27	[illegible]	6.86	0.70	0.16	9.99
S 75		32	1.43	7.9	8.07	0.65	0.14	7.43
S [illegible]		35	–	7.2	7.36	0.73	0.19	10.91
	Mean	34	1.33	7.4	7.24	0.67	0.14	7.83
	Range	32–37	1.22–1.40	7.2–7.9	6.38–8.07	0.54–0.77	0.05–0.24	5.11–10.91
S 44	Starvation for 24 hours	34	0.98	9.3	7.16	0.83	0.14	1.4
S 56		35	–	9.2	9.35	0.81	0.07	1.77
S 59		33	1.51	9.2	9.63	0.80	0.20	1.77
S 66		28	–	10.2	9.74	0.76	0.21	[illegible]
S 70		28	1.33	9.8	9.82	0.71	0.17	0.38
S 91		35	1.36	9.5	9.57	0.83	0.66	1.22
S 100		31		8.8	10.07	0.69	0.12	2.30
	Mean	32	1.30	9.4	9.33	0.77	0.23	1.55
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S 120	Starvation for 24 hours + 400 g glucose L	29	1.22	9.7	7.02	0.68	0.51	4.01
S 121		29	1.00	9.5	9.01	0.72	0.12	4.41
S 122		30	–	9.9	9.39	0.78	1.36	1.99
S 123		32	–	8.7	7.81	1.07	0.14	[illegible]
S 125		32	1.28	8.8	9.23	0.76	2.81	2.41
S 128		33	1.14	7.0	7.42	0.77	0.52	6.59
	Mean	31	1.16	8.9	8.32	0.80	0.91	3.86
	Range	29–33	1.00–1.28	7.0–9.9	7.02–9.39	0.58–1.07	0.12–2.81	1.99–6.59

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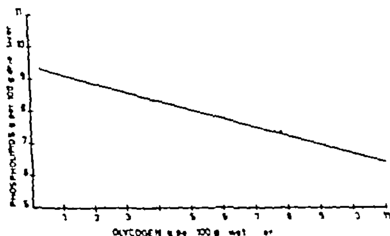


Fig. 2. Relationship between the concentration of phospholipids and glycogen in human liver tissue

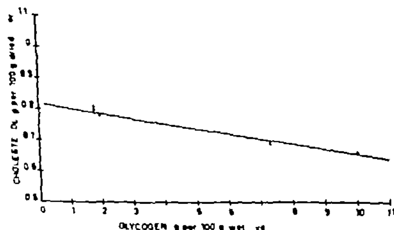


Fig. 3. Relationship between the concentrations of cholesterol and glycogen in human liver tissue

tent on one hand and the phospholipid and cholesterol contents on the other. These results are also given in table III and fig. 2 and 3.

Triglyceride content per 100 g dry liver substance

This varied between 0.12 and 0.54 g (average 0.33 g) on ordinary diet. In almost all patients in the investigation (24 out of 26) the triglyceride content was less than 0.7 g. No differences from this were observed in the 3 other experimental groups. Regression analysis showed no relationship between glycogen and triglyceride content.

D.N.A. content per 100 g dry liver substance

In the group on ordinary diet these values varied between 1.03 and 1.63 g (average 1.30 g). In the 3 other experimental groups the D.N.A. values were

Table III Statistical analysis of the relation between the content of glycogen, cholesterol, phospholipid and nitrogen

Groups compared	Equation of regression line	Correlation coefficient	P
Glycogen-nitrogen	y = 0.29x + 9 78	−0.78	< 0.001
Glycogen-phospholipid	y = 0.27x + 9.40	−0.61	< 0.001
Glycogen-cholesterol	y = 0.016 + 0.816	−0.43	< 0.05

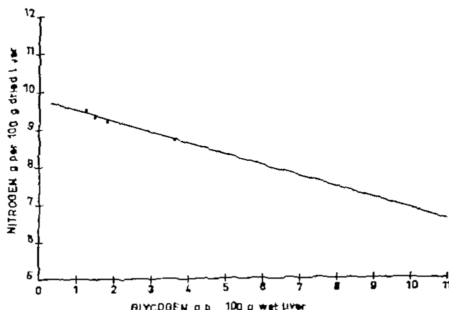


Fig 1 Relationship between the concentrations of nitrogen and glycogen in human liver tissue.

Nitrogen content per 100 g dry liver substance

In the group on ordinary diet the values for nitrogen varied between 7 0 and 11 3 g (average 8.9 g) The nitrogen values in the other three experimental groups were within the range of those in the group on ordinary diet. A tendency to higher nitrogen values was observed in the starved group at low liver glycogen values, and a tendency of low values in the group on ordinary diet + glucose. Statistical analysis showed a negative regression with a high nitrogen content at low glycogen content and vice versa. The results of the statistical analysis are given in table III and fig 1

Phospholipid and cholesterol content per 100 g dry liver substance

Phospholipid values varied between 6 17 and 9.58 g/100 g dry liver tissue (average 8.24 g) and cholesterol between 0.55 and 0 85 (average 0 77 g) in the groups on ordinary diet. The values for both these lipids in the three other experimental groups were practically all within the range of these in the group on ordinary diet.

There was the same tendency for these values as for nitrogen, namely high levels at a low glycogen content and vice versa On statistical analysis there was a negative regression between the glycogen con-

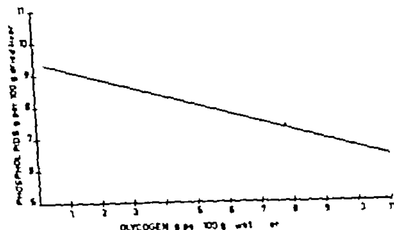


Fig. 2. Relationship between the concentration of phospholipids and glycogen in human liver tissue

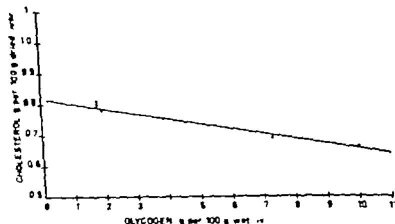


Fig. 3. Relationship between the concentrations of cholesterol and glycogen in human liver tissue

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Triglyceride content per 100 g dry liver substance

This varied between 0.12 and 0.54 g (average 0.33 g) on ordinary diet. In almost all patients in the investigation, (24 out of 26) the triglyceride content was less than 0.7 g. No differences from this were observed in the 3 other experimental groups. Regression analysis showed no relationship between glycogen and triglyceride content.

D.N.A. content per 100 g dry liver substance

In the group on ordinary diet, these values varied between 1.03 and 1.65 g (average 1.30 g). In the 3 other experimental groups the D.N.A. values were

Table IV The content of nitrogen, phospholipid, cholesterol and glycerides in relation to content of DNA

Patient	Experimental group	Nitrogen (g)	Phospholipids (g)	Cholesterol (g)	Triglycerides (g)
		Per g DNA			
S 41	Ordinary hospital diet	6.67	6.69	0.69	0.22
S 143		5.46	5.86	0.49	0.07
S 148		6.62	5.45	0.64	0.42
S 172		10.97	9.30	0.78	0.37
	Mean	7 43	6.83	0.65	0.27
	Range	5.46–10.97	5.86–9.30	0.49–0 78	0.07–0.42
S 63	Ordinary hospital diet + 400 g glucose i.v	5.90	6.61	0.52	0.10
S 67		5.57	5.25	0.44	0.08
S 73		5.67	5.40	0.55	0.13
S 75		5.52	5.64	0.45	0.10
	Mean	5 67	5.73	0.50	0.10
	Range	5.25–5.90	5.23–6.61	0.44–0.57	0.08–0.13
S 44	Starvation for 24 hours	9.49	7.31	0.86	0.11
S 59		6.09	6.38	0.53	0.13
S 78		7.26	7.27	0.53	0.13
S 91		6.99	7 04	0.61	0.49
	Mean	7 46	7.00	0.63	0.22
	Range	6.09–9 49	6.38–7.31	0.53–0.86	0.13–0.48
S 120	Starvation for 24 hours + 400 g glucose i.v	7.95	5.75	0.56	0.42
S 121		9.50	9 04	0.72	0.12
S 125		6.88	7.21	0.59	2.20
S 128		6.14	6.51	0.68	0.46
	Mean	7.62	7 73	0.64	0.80
	Range	6.14–9.50	5.73–9.04	0.59–0.72	0.12–2.20

practically all within the range of those in the group on ordinary diet.

Content of nitrogen phospholipid, cholesterol and triglycerides g per g D.N.A (table IV)

Calculated per g D.N.A. the liver contained in the group on ordinary diet 7 43 (5 46—10 97) g nitrogen 6 83 (5 86—9 30) g phospholipids 0 65 (0 49—0 78) g cholesterol 0.27 (0 07—0 42) g triglycerides. The corresponding values for the 3 other experimental groups were with the exception of one single patient all within the range of the untreated group The isolated definitely high value for triglycerides (2.20 g) was observed in a patient, who had starved and been given glucose.

Discussion

The value for nitrogen and lipid in liver biopsies taken from patients submitted to experimental manipulation or not, on the whole show a good agreement with earlier given "normal values in literature. Billing et al. (1 2) and Ralli et al (13 14) have investigated the

amount of total lipids in liver specimen taken from "normal" cases and have found values between 2.6—8.3 g total lipid per 100 g wet liver. The mean values in these 4 investigations were 5.7, 5.0, 4.98 and 4.72 g total lipid per 100 g wet liver tissue. However "normal" cases in some of these investigations (1, 2) included cases with various diseases as alcoholism, cancer etc.

In an investigation of this kind on clinical material the values obtained can evidently only give the relationship between the various components. In the absence of values for the total weight of the liver absolute values cannot be obtained. An indirect estimation of change in the absolute values may however be arrived at by relating to a reference substance present in all cells, if the amount of this substance would remain unchanged during different experimental conditions. This involves the assumption that the number of cells in the organ should be constant. It has been shown in the adult rat that prolonged fasting and prolonged consumption of protein-free diet (3) had no significant influence on the mean D.N.A. content of the liver. The D.N.A. content of the liver of the adult rat thus seems to be a suitable standard of reference for those liver constituents which vary with altered dietary conditions (4).

In the present investigation D.N.A. analyses were due to scarcity of material only done on a limited part of the clinical material as there was an insufficient amount of biopsy material. The D.N.A. content per 100 g dried weight varied between 0.98 and 1.63. As we have encountered no earlier investigations on the D.N.A. content of human liver comparison can only be made with findings in animals. Lovtrup and Roos (11) have with an identical technique found a D.N.A. content of rat liver of 1.32 and of rabbit liver of 1.10 mg/100 mg dried tissue. These data agree with the present results on human liver.

In the 3 experimental groups nitrogen, phospholipids, cholesterol and triglyceride in relation to D.N.A. were within the range of the distribution of the values of the group on ordinary diet with the exception of one patient. These results probably mean that absolute values of the component studied have remained unchanged. Thus acute short starvation and massive administration of glucose do not seem to cause changes in the total amount of nitrogen and lipids of the liver.

With the exception of 2 patients the triglyceride content was below 1 per cent. Earlier studies have shown higher values — around 2—3 per cent (6). These studies were made with another method of analysis, viz. by weighing of the petroleum-ether soluble fraction of an alcohol-ether extraction and the analyses were performed on liver samples taken at autopsy from patients, dead of various diseases.

The lack especially of consistent response in the neutral fat levels of the liver to the experimental changes is of interest and requests further investigation. Possibly studies of the relationships between serum and liver triglyceride might provide useful information. Such studies are in progress.

There is a negative regression between on one hand glycogen and on the other nitrogen, cholesterol and phospholipid. Our interpretation of the constant relationship between nitrogen, cholesterol and phospholipid on one hand and D.N.A. on the other during various experimental procedures is that the absolute values were unchanged. However slightly lower fig-

ures for nitrogen and the lipids per g D.N.A. were found in the group on ordinary diet + glucose. If our interpretation of the general constant relationship is correct the regression between the values of nitrogen, cholesterol and phospholipid in relationship to glycogen are explained solely by the shifts in glycogen Dry weight as reference value for various parameters of liver tissue can then only be used if the glycogen level is kept constant.

Summary

The effect was studied of short lasting acute starvation and intravenous administration of glucose on the liver content of glycogen nitrogen cholesterol phospholipid triglyceride and D.N.A. from biopsies taken at the beginning of operation in 26 patients with gastroduodenal ulcer The clinical material was divided into 4 groups one on ordinary diet, one on ordinary diet + glucose, one on starvation for 24 hours and one on starvation for 24 hours + glucose.

1 The average D.N.A. content of human liver tissue was 1 27 g per 100 g dried liver Values closely similar to these have been reported in animals.

2 The concentrations of nitrogen phospholid and cholesterol were inversely related to the concentration of glycogen.

3 There was no correlation between the concentration of triglycerides and any of the other parameters measured.

4 The concentrations of nitrogen phospholid cholesterol and triglyceride per g D.N.A. were not influenced by short acute starvation or by administration of glucose.

Acknowledgements

We are indebted to Dr K. Roos for performing the analysis of D.N.A. This work was partly supported by research grants from the Research Foundation of the Swedish Oleomargarin Trust.

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Coagulation Disturbances with Manifest Bleeding in Extrahepatic Portal Hypertension and in Liver Cirrhosis

Preliminary Results of Heparin Treatment

By

Eric Zetterqvist and Irene von Francken

It is well known that liver cirrhosis is often accompanied by deficiencies in the plasma content of several coagulation factors (16, 17 33) Increased fibrinolytic activity (1 15, 16) as well as thrombocytopenia (2, 31) and probably qualitative platelet changes (7 20) Increased capillary fragility has also been reported (34).

Lowered coagulation factor levels in liver parenchymal disease have been ascribed by most investigators to decreased synthesis (22, 30 32, 35) However in certain cases of liver cirrhosis the possibility of intravascular coagulation with consumption of factors has also been suggested (1) In this connection the finding of a high apparent activity of factor VIII (antihemophilic factor A, AHF) in cirrhosis and in metastatic liver disease is of interest (37) Furthermore in a bleeding patient with extrahepatic portal block with splenomegaly but without any demonstrable liver damage there was observed a high apparent AHF-activity in combination with decreased levels of the "liver-dependent" coagulation factors, as well as thrombocytopenia and a shortening of the coagulation time Although there is no direct evidence these findings suggest that consumption, rather than decreased synthesis, is the cause of low factor values at least in certain cases and that intravascular coagulation can be the primary cause of bleeding (37)

In this report are described two cases of portal hypertension in which the coagulation tests indicated the possibility of a hypercoagulable state which warranted therapeutic trials with heparin

Methods

Collection of blood samples. Blood was drawn with silicone technique as described by Nilsson et al. (24)

Whole-blood clotting time This was measured using plastic tubes, 10 cm long with an inner

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ures for nitrogen and the lipids per g D.N.A. were found in the group on ordinary diet + glucose. If our interpretation of the general constant relationship is correct the regression between the values of nitrogen, cholesterol and phospholipid in relationship to glycogen are explained solely by the shifts in glycogen. Dry weight as reference value for various parameters of liver tissue can then only be used if the glycogen level is kept constant.

Summary

The effect was studied of short lasting acute starvation and intravenous administration of glucose on the liver content of glycogen nitrogen cholesterol phospholipid triglyceride and D N.A. from biopsies taken at the beginning of operation in 26 patients with gastroduodenal ulcer The clinical material was divided into 4 groups one on ordinary diet one on ordinary diet + glucose one on starvation for 24 hours and one on starvation for 24 hours + glucose.

1 The average D.N.A. content of human liver tissue was 1 27 g per 100 g dried liver Values closely similar to these have been reported in animals.

2 The concentrations of nitrogen phospholid and cholesterol were inversely related to the concentration of glycogen.

3 There was no correlation between the concentration of triglycerides and any of the other parameters measured

4 The concentrations of nitrogen phospholid cholesterol and triglyceride per g D N.A. were not influenced by short acute starvation or by administration of glucose.

Acknowledgements

We ar indebted to Dr K. Roos for perfo the analysis of D.N.A. This work was partl ported by research grants from the Re Foundation of the Swedish Oleomargarin

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Table I Coagulation studies

Date	Coagulation time in plastic tube (min)	Recalcification time (sec pat./normal)	Factor VIII % of normal	Factor IX % of normal	Factor V % of normal	Prothrombin and proconvertin % of normal	Platelets × 10³	Fibrinogen (%)	Fibrinolysis µg hr pH 6.3	Fibrinolysis µg hr pH 7.1
Case 1										
9.3.62	8–12	273/202	140	86	54	60	110	0.31	500	251
19.3.62	3–7	164/172	350	84	72	80	108	0.41	60	0
23.3.62	6–14	–	–	–	–	–	176		–	–
10.4.62	3–9	–	500	108	103	87	78	0.23	[illegible]	0
25.4.62	11–18	202/182	156	76	127	69	53	0.30	3	0
Case										
22.2.62	–	155/160	649	113	95	52	131	0.30	40	17
27.2.62 (15^{00})	12	153/161	370	73	89	63		0.36	77	700
28.2.62 (18^{00})	35	704/223	410	87	95	61		0.35	7	4
28.2.62 (10^{00})	3	–	1160	–		–	65	–		–
28.2.62 (18^{00})	25	440/223	230	48	90	54		0.32	17	40
1.3.62 (11^{00})	16	246/183	383	73	78	69	90	0.35	0	227
1.3.62 (16^{00})	6	174/183	950	71	79	50	–	0.26	250	223
1.3.62 (23^{00})	60	478/183	405	48	71	54	68	0.29	27	27
2.3.62 (10^{00})	14	276/239	380	78	69	60	–	0.28	50	90
3.3.62 (9^{00})	13	228/183	365	49	90	61	52	0.27	73	120
4.3.62 (12^{00})	16	224/183	510	59	96	51	–	0.25	–	–
Normal value	18–33	–	100 ± 17.5	60–140	80–120	85–110	200–400	0.26 ± 0.06	31 ± 25	18–34

diameter of 9.5 mm. The tubes were filled to about two thirds with blood and the times for the fibrin threads to appear and for complete coagulation were noted. With this method we have found that normal blood coagulation begins after 17.5 min with a σ value of 5.1 and is complete after 32.9 min with $\sigma = 9.2$.

Recalcification time of plasma was measured as described by Nilsson et al. (24)

One stage prothrombin time was determined by the method of Quick using thromboplastin prepared from human brain.

Factor VIII (hemophilia A factor) was measured with the method described by Nilsson et al. (24)

Factor IX (hemophilia B-factor) was determined as described by Nilsson et al. (25)

Factor V (proaccelerin) was measured by the technique of Wolf (36)

Prothrombin and factor VII (proconvertin) This determination was made by the method of Owren and Aas (28)

Fibrinogen was determined by the method applied by Blombäck (3)

Fibrinolytic activity of plasma was determined by the method of Bergström et al. (1)

Case reports and coagulation studies

Case 1 A five year-old non-identical twin boy born prematurely (weight 1 700 g) Delivery was by caesarean section because of malignant pelvic tumor in the mother His development was normal until the age of 21 months, when episodes of profuse gastrointestinal bleeding began Splenomegaly and increased venous markings on the abdomen were demonstrated. Laparotomy gave no explanation of the bleeding Extrahepatic portal obstruction was suspected and the patient was transferred to a pediatric surgical clinic, where the following findings were made in 1959

Splenoportography extrahepatic obstruction of the portal vein at the hilum of the liver

X-ray of the esophagus varices in the lower part.

X ray of the abdomen moderately enlarged spleen.

Liver function tests no indications of liver damage.

At *operation* (gastric transection with ligation of collateral vessels) findings were

Mesenteric vein pressure 41 cm of water

Liver biopsy histologically normal liver

In 1961 after many further bleeding episodes the patient was again admitted to the special clinic. X ray showed renewed formation of a big net of collateral vessels in the fundus region of the stomach. Allison's operation was performed with the most effective ligation possible. Also on this occasion liver biopsy showed a histologically normal liver The bleeding continued postoperatively and the patient required a total of 6,000 ml of whole-blood during a period of three weeks. Because of suspected fibrinolysis epsilon-aminocaproic acid was given with a transient effect and the patient was free of bleeding for some weeks. Two months after this second operation esophagoscopy showed large varices.

The bleeding episodes continued and in 1962 the patient was again investigated and reconsidered for further surgery From the time of admission he had repeated episodes of profuse bleeding which for three weeks required an average daily transfusion of one pint of whole blood His weight was now 14 kg The hematological findings indicated bone marrow depression. Sternal marrow examination (Nordenson) showed a depressed myelopoiesis, a weak erythropoietic activity and depressed thrombopoiesis.

Coagulation studies (table I) indicated pathologically increased fibrinolysis, whereupon epsilon-aminocaproic acid was given with a prompt effect and clinically striking improvement. However heavy bleeding reappeared after a few days of treatment. As the coagulation time was extremely short it was decided to try treatment with heparin Epsilon-aminocaproic acid therapy was omitted and heparin was administered intravenously in a dose of 20 mg three times daily subsequently increased to 25 mg four times daily Following this the patient had with one exception only minor bleeding episodes for several weeks and his general state improved markedly The single major bleeding event occurred when subcutaneous heparin administration, 250 mg twice a day was tried in order to facilitate the therapy After two days of this regimen the heavy bleeding returned. When intravenous administration was resumed the patient again became free of bleeding symptoms within a few days.

Table I [illegible]

[illegible]	[illegible]	[illegible]	Fac. VIII (% of normal)	Fac. IX (% of normal)	Fac. X (% of normal)	[illegible]	[illegible]	[illegible]	[illegible]	
									[illegible]	[illegible]
Case I										
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	115	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	81	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0
[illegible]	[illegible]		–				[illegible]			
[illegible]	[illegible]		[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	0
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	0
[illegible]										
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11	0	[illegible]	[illegible]
[illegible]	12	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]		[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
[illegible]	[illegible]		[illegible]				[illegible]			
[illegible]	25	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		0.28	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.27	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	310	[illegible]	[illegible]	[illegible]		0.25		
Normal value	18–31	–	100 ± 17.5	60–140	80–120	85–110	200–400	0.75 [illegible]	31–25	18–38

diameter of 9.5 mm. The tubes were filled to about two thirds with blood and the times for the fibrin threads to appear and for complete coagulation were noted. With this method we have found that normal blood coagulation begins after 17.5 min with a σ value of 5 1 and is complete after 32 9 min with $\sigma = 9.2$.

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Coagulation studies (table I) indicated pathologically increased fibrinolysis, whereupon epsilon-aminocaproic acid was given with a prompt effect and clinically striking improvement. However heavy bleeding reappeared after a few days of treatment. As the coagulation time was extremely short it was decided to try treatment with heparin. Epsilon aminocaproic acid therapy was omitted and heparin was administered intravenously in a dose of 20 mg three times daily subsequently increased to 25 mg four times daily Following this the patient had with one exception only minor bleeding episodes for several weeks and his general state improved markedly The single major bleeding event occurred when subcutaneous heparin administration, 250 mg twice a day was tried in order to facilitate the therapy After two days of this regimen the heavy bleeding returned. When intravenous administration was resumed the patient again became free of bleeding symptoms within a few days.

[illegible] plasma [illegible]

[illegible] The [illegible] AHF-activity [illegible] normal [illegible] decreased [illegible] prothrombin [illegible] two pathological [illegible] coagulation [illegible] from [illegible] the first large haematemesis [illegible] factors were [illegible] normal [illegible] was [illegible]

[illegible] plasma [illegible] Ten hours [illegible] heparin dose of 100 mg [illegible] normal [illegible] further heparin [illegible] reduced to be [illegible] After another [illegible] hours the [illegible] was extremely [illegible] apparent AHF-activity was [illegible] (normal [illegible]) [illegible] The two [illegible] analyses [illegible] normal coagulation [illegible] heparin [illegible] Thirty-two hours after the last heparin dose the coagulation time was [illegible] apparent AHF-activity [illegible] pathological [illegible] demonstrable [illegible] 100 mg of heparin were [illegible] hours the dosage was changed to 25 mg of heparin every eight hours for nearly two days of this therapy the apparent AHF-activity remained at [illegible] of normal and the coagulation time [illegible] minutes. The last sample which was taken in connection with [illegible] large haematemesis [illegible] showed an increasing activity of apparent AHF and the values of factor V and prothrombin-proconvertin were somewhat decreased.

Discussion

In studies on the plasma fibrinolytic activity in a case of liver cirrhosis, Bergström, Blombäck and Kleen found some evidence for intravascular coagulation rather than fibrinolysis [illegible] hypercoagulable state in the patient [illegible] Their proposal was based on the fact that the fibrinogen [illegible] could be [illegible] plasma [illegible] all the normal [illegible] [illegible] liver cirrhosis [illegible]

In a previous [illegible] apparent AHF-activity [illegible] in liver cirrhosis [illegible]

High AHF-values have been reported [illegible] the presence of [illegible] normal [illegible] ACTH [illegible] these high activities [illegible] referred [illegible] report [illegible] use the term [illegible] apparent AHF-activity.

The findings [illegible] with the [illegible] apparent AHF [illegible] combined with a marked [illegible] coagulation time [illegible] platelets [illegible] that such increased AHF-activity could be [illegible] expression of hypercoagulability [illegible] The [illegible] that [illegible] mechanism [illegible] important [illegible] bleeding has been supported by [illegible] effects obtained with heparin administration in certain postoperative bleeding states of [illegible] as well as by observations in a case of liver cirrhosis made independently by Johansson (1).

In this connection it might be mentioned that heparin treatment has also been given with good clinical effect in other diseases, where coagulation studies indicated a hypercoagulable state (3, 21, 29).

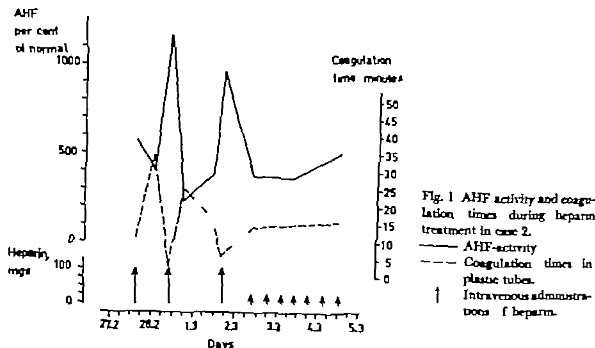


Fig. 1 AHF activity and coagulation times during heparin treatment in case 2.
—— AHF-activity
– – – Coagulation times in plastic tubes.
↑ Intravenous administrations f heparin.

The first *coagulation analysis* (table I) after a long period of bleeding showed a shortening of the coagulation time, a nearly normal AHF-activity reduced levels of factor V (proaccelerin) and prothrombin-proconvertin, and a clearly abnormal increase of fibrinolytic activity (9 3 62) Ten days later (19 3 62) when bleeding had ceased after three days of treatment with epsilon aminocaproic acid, the coagulation time was strikingly short, the apparent AHF activity was 550 of normal and the levels of factor V and prothrombin proconvertin were normal furthermore, there was no increased fibrinolytic activity After the change to heparin treatment the coagulation time was clearly shortened six hours after the first dose of 20 mg insufficient blood was obtainable for other tests (23 3. 62) Heparin treatment was continued, but because of the age of the patient and the difficulties of obtaining blood samples further studies were not performed until three weeks later (10. 4 62) when the trial of subcutaneous heparin treatment had been made. The analyses now showed a shortening of the coagulation time and an apparent AHF-activity 500 of normal the levels of other factors were normal. Intravenous administration of heparin was again started. After another two-week period during which the patient was free of bleeding the apparent AHF-activity was 156 prothrombin proconvertin was 69 and the other factors tested were normal no fibrinolysis could be demonstrated and the coagulation time was 11—18 minutes (25. 4 62)

Case 2 This patient was a 52 year-old man with a history of alcohol abuse for about 30 years, increasing in severity during the last years. In August 1961 he complained of dull pain below the right costal margin, marked tiredness and nausea. Episodes of small hematemesis and a tendency to epistaxis subsequently began. Five months after the first symptoms the patient developed jaundice and ascites. Liver biopsy showed advanced Laënnec cirrhosis. X-ray examinations showed large esophageal varices and splenomegaly Electrophoretic findings and other laboratory values were consistent with the diagnosis of liver cirrhosis. Hematological studies showed thrombocytopenia findings were otherwise normal. The patient was treated with corticosteroids and diuretics and his ascites subsided. After five weeks of treatment, during which period he showed no bleeding manifestations, the patient suddenly had a large hematemesis. In vitro coagulation tests immediately after this episode indicated hypercoagulability and 100 mg of heparin were given intravenously The heparin treatment (dosage given in fig 1) was continued for five days thereupon

Table II Coagulation factors in liver cirrhosis and in metastatic liver disease

	Cirrhosis			Metastatic liver			Normal value
	No. of investigat.	Mean value	Range	No. of investigat.	Mean value	Range	
Factor VIII (antihemophilic factor A, AHF) (%)	47	337	120–790	11	345	135–897	100 ± 17.5
Factor IX (antihemophilic factor B) (%)	37	66	33–115	11	91	18–183	60 – 140
Factor V (proaccelerin (%)	38	64	31–173	10	114	50–158	80 – 120
Prothrombin and proconvertin (%)	38	58	33–130	11	65	13–97	85 – 110
Fibrinogen (%)	38	0.30	0.15–0.73	7	0.70	0.21–1.2	0.26 ± 0.06
Fibrinogen at pH 6.3 (µg/hr)	42	73	0–705	7	49	0–100	31 ± 25
Fibrinolysis at pH 7.1 (µg/hr)	41	41	0–626	7	88	0–307	18 ± 34
Platelets (/mm³)	40	113 000	20,000–311 000	15	169 000	49 000–290 000	200,000–400 000

In the present investigation some observations especially on the first patient, who was studied over a period of several weeks, are in favour of increased coagulation *in vivo* as a primary cause of the bleeding The patient had during three weeks suffered from heavy bleeding which had required daily transfusions with an average of one pint of whole blood The initial therapy with epsilon aminocaproic acid was efficient only during the first four days. Despite treatment bleeding reappeared but stopped promptly after intravenous administration of heparin in doses similar to those used in the treatment of thrombosis. In a few days the patient became almost free of bleeding symptoms and began to recover He then had only small melaenas for a period of seven weeks with one exception which occurred when subcutaneous heparin administration was instituted. After resumption of intravenous administration the patient again became free of bleeding symptoms within a few days. Two weeks later analyses showed a nearly normal coagulation pattern and no fibrinolysis. Most probably the subcutaneous heparin administration had been inadequate.

The coagulation pattern of the second patient before heparin administration was started showed essentially the same changes as that of the first, shortening of the coagulation time and elevated apparent AHF activity being the predominant features. These findings were interpreted as indications of hypercoagulability

As can be seen from fig there was a tendency toward normalization of the

coagulation pattern during heparin treatment. Although the samples were drawn six hours or more after the heparin injection, some influence of remaining heparin upon the determinations of factors VIII and IX cannot be excluded. However, Eiselack et al. have shown that six hours after an intravenous heparin dose of 100 mg to a normal person weighing 75 kg the heparin content in plasma would be not more than 0.1—0.2 IU per ml plasma (6).

Regarding the clinical effect we would like to emphasize the fact that a heparin injection of 100 mg immediately after a major hematemesis was not followed by increased bleeding. This observation is also in accordance with our experience from other cases of the same type. In fact the patient did not show any signs of threatening hemorrhage during the five days of treatment until the last fatal hematemesis occurred.

In these cases the variations in the fibrinolytic activity as measured according to Bergstrom et al (1) do not seem to be well correlated to the occurrence of bleeding, an observation which is in accordance with the findings in other investigations (…). The striking clinical effect of epsilon aminocaproic acid in the first patient however does not exclude fibrinolysis as a cause of increased bleeding. It is possible that fibrinolysis, in cases like these, is secondary to a state of hypercoagulability. If so, treatment with epsilon-aminocaproic acid should not be the first choice. It may be remarked in this connection that the possibility of thrombous development complicating therapy with this substance has recently been mentioned (23).

Summary

Reports are given on two cases of portal hypertension with bleeding complications where heparin treatment was tried with a good clinical effect. Coagulation findings before treatment in both patients indicated the possibility of hypercoagulability as the primary coagulation disorder. The essential changes were:

1. A shortened coagulation time as measured in plastic tubes, and

2. A high apparent activity of factor VIII (antihemophilic factor A, AHF).

Acknowledgement

This investigation was aided by … from the Swedish Medical Research Council.

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Fibrinogen (%)	38	0.30	0.15—0.73	7	0.70	0.21—1.2	0.26 ± 0.06
Fibrinogen at pH 6.3 (μg/hr)	42	73	0—705	7	49	0—100	31 ± 25
Fibrinolysis at pH 7.1 (μg/hr)	41	41	0—626	7	88	0—307	18 ± 34
Platelets (/mm³)	40	113 000	20 000—311 000	15	169 000	49,000—290,000	200,000—400,000

In the present investigation some observations especially on the first patient, who was studied over a period of several weeks, are in favour of increased coagulation *in vivo* as a primary cause of the bleeding. The patient had during three weeks suffered from heavy bleeding which had required daily transfusions with an average of one pint of whole blood. The initial therapy with epsilon aminocaproic acid was efficient only during the first four days. Despite treatment bleeding reappeared but stopped promptly after intravenous administration of heparin in doses similar to those used in the treatment of thrombosis. In a few days the patient became almost free of bleeding symptoms and began to recover. He then had only small melaenas for a period of seven weeks with one exception which occurred when subcutaneous heparin administration was instituted. After resumption of intravenous administration the patient again became free of bleeding symptoms within a few days. Two weeks later analyses showed a nearly normal coagulation pattern and no fibrinolysis. Most probably the subcutaneous heparin administration had been inadequate.

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As can be seen from fig. there was a tendency toward normalization of the

coagulation pattern during heparin treatment. Although the samples were drawn six hours or more after the heparin injections, some influence of remaining heparin upon the determinations of factors VIII and IX cannot be excluded. However Blombäck et al. have shown that six hours after an intravenous heparin dose of 100 mg to a normal person weighing 75 kg the heparin content in plasma would be not more than 0.1—0.2 IU per ml plasma (6).

Regarding the clinical effect we would like to emphasize the fact that a heparin injection of 100 mg immediately after a major hematemesis was not followed by increased bleeding. This observation is also in accordance with our experience from other cases of the same type. In fact, the patient did not show any signs of threatening hemorrhage during the five days of treatment until the last fatal hematemesis started.

In these cases the variations in the fibrinolytic activity as measured according to Bergström et al. (1) do not seem to be well correlated to the occurrence of bleeding, an observation which is in accordance with the findings in other investigations (1). The striking clinical effect of epsilon-aminocaproic acid in the first patient however does not exclude fibrinolysis as a cause of increased bleeding. It is possible that fibrinolysis, in cases like these is secondary to a state of hypercoagulability. If so, treatment with epsilon-aminocaproic acid should not be the first choice. It may be remarked in this connection that the possibility of thrombosis development complicating therapy with this substance has recently been mentioned (23).

Summary

Reports are given on two cases of portal hypertension with bleeding complications, where heparin treatment was tried with a good clinical effect. Coagulation findings before treatment in both patients indicated the possibility of hypercoagulability as the primary coagulation disorder. The essential changes were

1. A shortened coagulation time as measured in plastic tubes, and

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Acknowledgement

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Byssinosis

Differential Diagnosis from Bronchial Asthma and Chronic Bronchitis

By

H. Arvoldsson, A. Bouhuys and S. E. Lindell

It has been known for over a century that many cotton mill workers complain of dyspnea during their work. This has been confirmed in several investigations, reviewed by Schilling (18). It is now generally agreed that a disease entity usually called byssinosis, occurs among cotton and other textile workers. The disease is presumably caused by inhalation of airborne dust during the day's work.

Byssinosis occurs among workers in cotton mill card rooms, the department where raw cotton is spun into a crude thick lint before being transferred to the spinning machines. These workers complain of dyspnea, increasing during the course of the day's work, and sometimes accompanied by general malaise. The complaints usually disappear within some hours after the end of work. A peculiar characteristic is that dyspnea mainly occurs on first working days after a period of absence from work, such as a week end or a vacation, therefore mainly on Mondays and not, or much less, on other weekdays. Many byssinotic workers indicate that their troubles are the worse the longer they have been away from the card rooms. Only after many years of exposure do the symptoms acquire a chronic character and then they persist even if the worker changes his occupation. The patients may thus be entirely free of symptoms on days they have not been in the card room and also during the last days of the working week in spite of exposure to card room dust. Clinical signs, when present, may be indistinguishable from those of bronchial asthma or chronic bronchitis. X-ray examination of the lungs discloses no characteristic changes. Spirometry may give normal values, unless linked with exposure to card room dust early in the working week.

The *diagnosis* is therefore based on 1. A history of exposure to the dust in the card room of a cotton mill and dyspnea which is most pronounced after a free week-end or holiday. 2. Measurements of changes in lung ventilation in the course of the day's work.

Table 1 Respiratory function in byssinotic patients before and after work

Case	Age (yrs)		Vital capacity (l BTPS)	Forced expiratory vol 1.0 (l BTPS)	Forced expiratory vol.1.0/ Vital capacity (%)	Maximal voluntary ventilation (l/min BTPS)	Single breath N % diff.	N_2-washout time (min)	Functional residual capacity (l BTPS)	Lung clearance index
1	55	Before	3.6/ 4 4	1 6/ 3 1	45/ 72	56/ 137	6.5/ 5.5	—	—	—
		After	2.7	0.9	35	34	6.5	—	—	—
		Before	2.8	1 4	50	—	7	5.95 5.27	4.3/ 3.2 3.7	11.1 12.2
		After	2 4	1 0	42	—	10	8.02 7 45	5.1 5.1	13.6 12.8
2	64	Before	5.1/ 3.5	2.0/ 2.3	64/ 68	67/ 123	2.2	1 18 1 75	3.1/ 3.3 3.2	8.4 6.9
		After	2.2	1.3	60	—	2.6	4.20 2.55	3.4 3.4	9.1 9 4
3	61	Before	2.3/ 4.5	1.5/ 3 1	62/ 69	55/ 128	—	—	—	—
		After	1.5	0.7	47	33	—	—	—	—

Denotes predicted normal values.
In the single breath study the nitrogen concentration at 750 and 1,200 ml exhaled air was measured differences are given as the average of 3 measurements.

We have recently had the opportunity to study three patients with byssinosis, two of them being referred to this hospital with a diagnosis of bronchial asthma.

Case reports

Case 1 The patient, a male of 55 who had been employed as a carding machine fitter since 1938, was sent to this clinic in 1960 for suspected bronchial asthma. Ascribes the complaints, which were said to have started in 1953, to exposure to cotton dust. Two hours after starting to work on Mondays, or the first working day after a holiday or va cation unproductive coughing d spnea and some wheezing set in. The dyspnea is described as a feeling of oppression in the chest. For the past two years the symptoms occur on other weekdays but never on holidays or during vacations and he has had moderate exer tional dyspnea.

Physical and laboratory findings Heart no physical abnormalities. Blood pressure 150/ 100 mm Hg ECG at rest and during exercise no abnormalities. Lungs some dry râles over basal regions. Chest X-ray heart and lungs normal ESR 7 mm/hour Eosinophils normal in blood none in sputum Skin tests with various allergens negative Provocation tests by inhalation of extracts of dusts, moulds and cottonseed negative After a few days absence from work, an hour exposure to cotton dust in the card room induced asthma like symptoms of the type described above Results of lung function studies are given in table I

Case 2. This patient, a male aged 64, had been working in a cotton mill card room for the last 15 years. His health had been good until 7 or 8 years ago, when he started to have asthma-like symptoms with coughing and dyspnea on Mondays some hours after starting work. There was no expectoration. Symptoms were at first absent on other week days and were particularly bad after week ends or vacations. After some years they occurred also on other weekdays, and during the past year the symptoms became more like those in chronic obstructive lung disease. The complaints were always worse during work, particularly after absence from work.

Physical and laboratory findings. General health satisfactory. Lungs slightly lowered margins. Heart no physical abnormalities. Blood pressure 150/100 mm Hg. ECG normal. Chest X-ray: heart and lungs normal. ESR 5 mm/hour. Eosinophils normal in blood, none in sputum. Skin tests with various allergens and provocation tests with extracts of dust, moulds and cottonseed, negative. Results of lung function studies are given in table I.

Case 3. The patient, a male of 61, had been working in a cotton mill card room since 1930. Up to the onset of present disease he had been in good health. Visiting the Clinic in Sept. 1961 for asthma-like disease, he complained of coughing, dyspnea and some wheezing after working 1 or 2 hours on Mondays since 1950. There was no expectoration. The complaints had gradually become worse and during the past year had started to occur on Tuesdays too. Symptoms are absent on other weekdays and during absences from work. After vacations the symptoms were particularly troublesome, and occasionally dyspnea had forced him to stop working. The symptoms have been absent when he has been working away from the card room. During the past year exertional dyspnea has supervened.

Physical and laboratory findings. General health satisfactory. Heart no physical abnormalities. Blood pressure 160/90 mm Hg. ECG at rest and during exercise normal. Lungs: few dry rales over basal regions. Chest X-ray: heart and lungs normal. ESR 12 mm/hour. Eosinophils normal in blood, none in sputum. Skin tests with various allergens and provocation tests with extracts of dusts, moulds and cottonseed, negative. Results of lung function studies are given in tabl I.

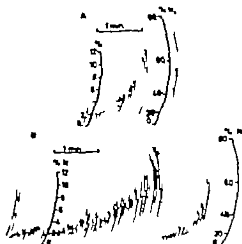


Fig. 1. Nitrogen clearance before work (A) and at the end of 6 hours work in the card room (B) in case 2. Read from right to left. Records made during quiet breathing at rest.

A clinical diagnosis of byssinosis was made in all three cases.

Methods

In order to assess more objectively the changes in pulmonary function in these subjects during the working day measurements of pulmonary ventilation were made. The workers were examined before and about half an hour after the end of their usual work in the card room (about 6 hours exposure). They had been away from the card room 36 hours before the first measurement. The following methods were used:

1. Determination of vital capacity (V.C.), maximal voluntary ventilation (M.V.V.) and forced expiratory volume (F.E.V.) with a spirometer similar to that described by Bernstein et al. (2) and Grimby and Söderholm (12).

2. Determination of the slope of recorded curves of nitrogen concentration in expired air after a single oxygen inspiration, according to the method of Birath (3).

3. Determination of washout time, functional residual capacity and lung clearance index, using the open-circuit nitrogen meter technique, described by Bouhuys et al. (5).

Results

The results are summarized in table I which shows that none of the subjects had an entirely normal ventilation before exposure to dust. After 5 to 8 hours of work in the card room all three subjects showed a decrease in vital capacity of obstructive character At the same time the distribution of inhaled gas became more uneven as judged from the washout of nitrogen *during oxygen breathing* studies with the multiple breath method (fig 1) With the single breath method the changes were less conspicuous

Discussion

Two subjects (cases 1 and 3) came to the hospital with a diagnosis of bronchial asthma Their work in a cotton mill card room aroused suspicion that they actually had byssinosis. Both spontaneously gave a history of respiratory distress during the working day particularly on Mondays or other days after absence from work. Such a history is considered characteristic of byssinosis (18 19) Changes in respiratory function during the working day were also shown by the lung function tests.

Chest X rays showed no definite abnormalities There was no demonstrable skin sensitivity to common antigens, nor to dust from the card rooms where they worked There was no demonstrable reaction in bronchial sensitivity tests with the common inhalants

These findings suggest that the symptoms were due to byssinosis.

Byssinosis has been found to occur in cotton mills in England (18) The Netherlands (22) Belgium (Verbeke personal communication) France (21) U.S.A. (4) and India (20) In 1953 Dalhamn and Ekberg found no symptoms of byssinosis in 40 Swedish cotton workers most of them with normal lung function tests. They did not report studies of possible changes in lung ventilation during the course of a day's work. Only few investigations have dealt with the actual prevalence of byssinosis among workers exposed to cotton dust in card rooms. Schilling (18) reported a prevalence as high as 60 % among some groups of workers. The prevalence may to some extent depend on the quality of the cotton and on measures taken to remove dust from the card rooms. However there is some evidence that technically adequate dust removal is in itself insufficient to abolish the workers complaints (18) The prevalence of byssinosis will probably be underestimated if judged only from the history Many workers are so accustomed to their Monday dyspnea that they do not mention it spontaneously Several investigators have mentioned the occurrence of byssinosis among flax workers (21) In a recent study of flax workers (8) several workers were seen who although not giving a history of Monday dyspnea, showed a clear decrease of ventilatory capacity during the day s work on Monday and were clearly dyspneic at the end of the working day Therefore, unless accompanied by studies of changes in respiratory function during the working day reports on byssinosis appear to be of limited value

McKerrow et al (15) found that the ventilatory capacity of byssinotic card room workers decreased in the course of the day s work, particularly on Mondays. This decrease was largely reversible, as shown by the results on other weekdays. The ventilatory capacity was assessed by the Gaensler technique (direct reading of F E $V_{0.75}$, using a spirometer with a timing device described by McKerrow

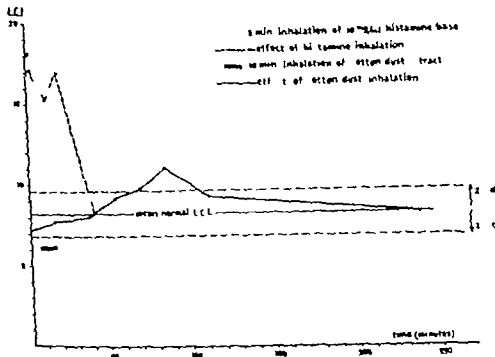


Fig. 2. Time course of the effects of inhalation of histamine aerosol and of cotton dust extract aerosol in healthy male subject, as estimated from changes in lung N_2 clearance during oxygen breathing

et al. (15). This method requires some active cooperation of the subjects in performing maximally rapid expirations. However by showing that the gas flow resistance of the airways increased during the working day McKerrow et al. (15) could confirm their findings with an objective method. These workers also showed that adrenaline inhalation at the end of the day's work increased the ventilatory capacity.

In the later stages of the disease the ventilatory changes no longer disappear after the end of work, often making the syndrome difficult to distinguish from other forms of chronic obstructive lung disease. Although the pathogenesis of byssinosis is not fully understood recent investigations have shed some light on this much debated problem. The respiratory function studies mentioned above indicate that the Monday dyspnea of byssinosis is caused by reversible bronchial constriction, i. e. bronchospasm, edema of the bronchial mucosa, bronchial secretions, or any combination of these factors. It has generally been assumed that these changes are caused by inhalation of cotton dust, but how this acts on the bronchi has been a controversial subject.

In a previous study Bouhuys et al. (6) found that inhalation of an extract of cotton dust obtained from a card room, produced dyspnea and general malaise on first exposure in healthy human subjects. Changes in the ventilation of the lungs were demonstrated objectively by the nitrogen clearance technique de

Results

The results are summarized in table I which shows that none of the subjects had an entirely normal ventilation before exposure to dust. After 5 to 8 hours of work in the card room all three subjects showed a decrease in vital capacity of obstructive character At the same time the distribution of inhaled gas became more uneven as judged from the washout of nitrogen during oxygen breathing studies with the multiple breath method (fig 1) With the single breath method the changes were less conspicuous.

Discussion

Two subjects (cases 1 and 3) came to the hospital with a diagnosis of bronchial asthma Their work in a cotton mill card room aroused suspicion that they actually had byssinosis. Both spontaneously gave a history of respiratory distress during the working day particularly on Mondays or other days after absence from work. Such a history is considered characteristic of byssinosis (18 19) Changes in respiratory function during the working day were also shown by the lung function tests.

Chest X rays showed no definite abnormalities There was no demonstrable skin sensitivity to common antigens, nor to dust from the card rooms where they worked There was no demonstrable reaction in bronchial sensitivity tests with the common inhalants.

These findings suggest that the symptoms were due to byssinosis.

Byssinosis has been found to occur in cotton mills in England (18) The Netherlands (22) Belgium (Verbeke, personal communication) France (21) U S.A. (4) and India (20) In 1953 Dalhamn and Friberg found no symptoms of byssinosis in 40 Swedish cotton workers, most of them with normal lung function tests. They did not report studies of possible changes in lung ventilation during the course of a day's work. Only few investigations have dealt with the actual prevalence of byssinosis among workers exposed to cotton dust in card rooms. Schilling (18) reported a prevalence as high as 60 % among some groups of workers. The prevalence may to some extent depend on the quality of the cotton and on measures taken to remove dust from the card rooms. However there is some evidence that technically adequate dust removal is in itself insufficient to abolish the workers complaints (18) The prevalence of byssinosis will probably be underestimated if judged only from the history Many workers are so accustomed to their Monday dyspnea that they do not mention it spontaneously Several investigators have mentioned the occurrence of byssinosis among flax workers (21) In a recent study of flax workers (8) several workers were seen who although not giving a history of Monday dyspnea, showed a clear decrease of ventilatory capacity during the day's work on Monday and were clearly dyspneic at the end of the working day Therefore, unless accompanied by studies of changes in respiratory function during the working day reports on byssinosis appear to be of limited value.

McKerrow et al (15) found that the ventilatory capacity of byssinotic card room workers decreased in the course of the day's work, particularly on Mondays. This decrease was largely reversible as shown by the results on other weekdays. The ventilatory capacity was assessed by the Gaensler technique (direct reading of $F E V_{0.75}$, using a spirometer with a timing device described by McKerrow

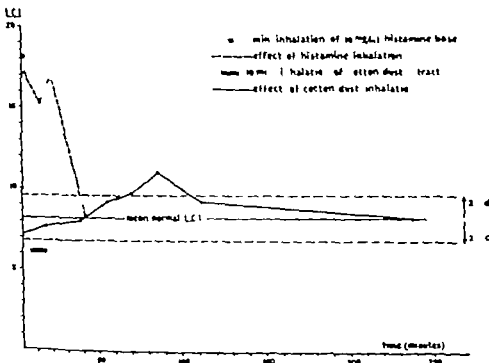


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scribed by Bouhuys et al. (5) A second inhalation next day had no effect either subjectively or on the nitrogen clearance of the lungs.

Several conclusions may be drawn from these results. Firstly the fact that an effect is obtained on first exposure to the dust extract indicates that it is independent of previous sensitization to some dust constituent It thus does not seem to be an allergic reaction in the usual sense, as postulated by Haworth and McDonald (13) Secondly the effect of the cotton dust in the mills is probably caused not by mechanical irritation of the bronchi by dust particles, because a similar effect was induced by inhaled cotton dust extract and furthermore, inhalation of an otherwise similar extract of house dust had no effect on the bronchi The evidence so far favours the assumption that cotton dust contains an active pharmacological agent which causes bronchoconstriction by any of the mechanisms mentioned above

Finding a histamine-like activity in cotton dust McDonald and Maitland (11) suggested that histamine might play a role in the etiology of byssinosis. It is well known that histamine inhalation can induce bronchoconstriction in normal subjects and cause dyspnea. However the histamine content of active cotton dust extract is too small (less than 1 $\mu g/g$ dust) for it to be the cause of dyspnea in byssinotics. In normal subjects the histamine concentration in nebulized fluid must be about 5 mg/ml or more to produce clear effects (7) For these reasons, it may be excluded that the presence of histamine in the dust is responsible for the symptoms of dyspnea.

The effect of cotton dust extract on normal subjects differs from the effect of inhaled histamine (fig 2) whereas the cotton dust extract has its maximal effect after about one or two hours, histamine has a maximum effect during or directly after inhalation

Thus the effect of cotton dust resembles that of histamine in so far as both cause bronchoconstriction. Yet the effect of cotton dust cannot be explained either by the presence of histamine itself or by histamine released in an antigen-antibody reaction. However there remains a third possibility histamine release by some chemical compound in the cotton dust. The work of McIntosh and Paton (14) has shown that many chemical substances are capable of releasing histamine in tissues without the intervention of a preceding antigen-antibody reaction On the basis of inhalation experiments in man the hypothesis has been advanced that cotton dust contains a substance capable of releasing histamine in the human lung (6) Depletion of releasable histamine in the lungs after the first dust exposure could explain the absence of an effect of repeated exposures. This hypothesis could also explain the longer latency and the longer duration of the effect, compared with that of histamine itself. Moreover the hypothesis also accounts for the fact that dyspnea in byssinotic workers is the worse the longer the absence from work.

Animal experiments have produced evidence of the presence of a histamine releasing substance in cotton dust (1 6 17) Davenport and Paton (10) found that cotton dust extracts released some histamine in rats, but not in cats or guinea pigs. *In vitro* histamine is released from human lung tissue incubated with cotton dust extract at body temperature (8)

Accordingly the symptoms of byssinosis may at least partly be explained by the presence of a histamine-releasing sub-

tance in cotton dust. In particular this could account for the fact that card room workers have their complaints mainly on the working-day after a leave of absence, assuming that the releasable histamine in the bronchial walls is depleted during the first working-day and that exposure to the dust must be interrupted temporarily before the subject again becomes sensitive to the dust effect. Several workers have told us that the longer they are away from work the more severe their dyspnea on the first working-day And this also fits into this explanation

So far the histamine-releasing substance in cotton dust has not been identified It seems highly improbable that the cotton fibres themselves have such an activity since cellulose is known to be an inert substance in many respects. Baled raw cotton contains many impurities, such as plant residues, and is often stored for long periods before being spun It usually contains many bacteria and fungi, and the histamine-releasing agent could be some product of bacteria or fungi. Among flax workers byssinosis has been found only in those who come into contact with the dust of retted flax, i. e. flax which has been subjected to bacterial action (8)

Until prevention becomes possible, the best treatment for byssinosis must be to remove the patient from exposure to the dust. The use of a mask with dust filters does not seem to prevent byssinosis although the symptoms are ameliorated. Treatment with antihistamines has been found to decrease but not eliminate the changes in pulmonary ventilation caused by the dust in the card room. At present the symptomatic of choice seems to be isoprenaline given by inhalation (Bouhuys, unpublished)

The course is not definitely known but the incidence of chronic respiratory insufficiency among elderly cotton workers is said to be fairly high. It should be noted that none of the subjects in this study had an entirely normal pulmonary ventilation, even though they had been away from work at least 36 hours before spirometry The impairment of pulmonary ventilation recorded initially was probably due to some obstructive pulmonary disease secondary to byssinosis.

Summary

Byssinosis is a disease entity affecting some groups of cotton flax and hemp workers. The main symptoms are dyspnea and cough. In the early stages of the disease, these occur only on the first working day after a week-end or a holiday i. e usually on Mondays. The symptoms of byssinosis resemble those of bronchial asthma or chronic bronchitis, which may call for a differential diagnosis of these three diseases.

Three cases are reported in which byssinosis was diagnosed clinically in cotton mill workers. The disease seems to be more prevalent than previously supposed. The course of byssinosis is fairly characteristic in the early stages, but studies on the disease appear to be of little value unless they are accompanied by measurements of respiratory function during the day's work.

The pathogenesis of byssinosis is imperfectly understood, and how the card room cotton dust acts in the bronchi is a moot point. A number of possible causative factors are discussed. The reaction is apparently not an allergic one in the usual sense and is probably not induced by mechanical irritation. The presence in cotton dust of a histamine releasing substance could explain the bronchoconstrictive symptoms of byssinosis.

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Several conclusions may be drawn from these results. Firstly the fact that an effect is obtained on first exposure to the dust extract indicates that it is independent of previous sensitization to some dust constituent. It thus does not seem to be an allergic reaction in the usual sense, as postulated by Haworth and McDonald (13) Secondly, the effect of the cotton dust in the mills is probably caused not by mechanical irritation of the bronchi by dust particles, because a similar effect was induced by inhaled cotton dust extract and furthermore, inhalation of an otherwise similar extract of house dust had no effect on the bronchi. The evidence so far favours the assumption that cotton dust contains an active pharmacological agent which causes bronchoconstriction by any of the mechanisms mentioned above

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The Reaction of the Pituitary-adrenocortical System to Stress after Prolonged Corticosteroid Therapy

By

H. Arnoldsson and L. Helander

Long-term corticosteroid therapy is generally agreed to reduce the endogenous steroid production of the adrenal cortex. This reduction is probably due to inhibition of the synthesis of endogenous ACTH when corticosteroids are being administered continuously Holub et al. (10) studied a series of patients who had undergone prolonged corticosteroid treatment and inferred that the release of ACTH apparently was diminished in all patients during the period of therapy and probably for a variable length of time after its cessation. However the occurrence in some patients of pituitary ACTH depletion indicated that not only the release but also the synthesis of ACTH had been impaired. Other investigators, such as Carreon et al. (5) and Kyle et al. (12) Anderson and Kjerulf (2) have also expressed the view that pituitary and not adrenal suppression is the primary difficulty in patients undergoing prolonged corticosteroid medication.

According to some authors, when hypocorticodism is in fact induced by long-term administration of exogenous corticosteroids there may be serious consequences. It has been pointed out, for example that in some patients interruption of corticosteroid administration gives rise to vertigo nausea, vomiting and fatigue for a few days. Henneman et al. (9) applied the term "withdrawal syndrome" to these signs and symptoms, but others (1 10) doubt that they are induced by hypocorticodism. Again others (1 15) have reported cases in which adrenocortical deficiency secondary to corticosteroid therapy has been held responsible for deaths following surgical interventions or other situations of stress.

Administration of exogenous ACTH has long been used in attempts to reduce the risks of such emergencies after predictable stress situations and interruption of long-term corticosteroid therapy Some workers have proposed that all patients undergoing long-term corticosteroid therapy routinely should be given exogenous ACTH every week or two (4) The objection has been raised, however

Acknowledgement

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Table I. Urinary excretion of 17-ketogenic and 17-ketosteroids in patients with bronchial asthma, before during and after induced fever

Day	Treatment	Patients not previously treated with corticosteroids: Rectal afternoon temperature, °C — Mean	Range	Urinary excretion of steroids in % of basal values: 17-ketogen — Mean	S. E. of mean	17-keto — Mean	S. E. of mean	Patients previously treated with corticosteroids: Rectal afternoon temperature, °C — Mean	Range	Urinary excretion of steroids in % of basal values: 17-ketogen — Mean	S. E. of mean	17-keto — Mean	S. E. of mean
1 (Basal values)	—	37.3	37.0–37.5					37.3	37.0–37.5				
2 (Basal values)	—	37.3	36.9–37.7	100	—	100	—	37.3	36.8–37.6	100	—	100	—
3 (Basal values)	—	37.3	36.8–37.4					37.2	36.9–37.4				
4	Induced fever	38.9	38.3–39.7	197	±36	163	±17	39.5	38.8–40.5	160	±33	150	±13
5	—	37.6	37.1–38.1	102	±11	110	±21	37.6	36.8–38.6	125	±25	142	±28
6	—	37.3	36.7–37.7	78	± 8	97	±17	37.2	36.8–37.4	109	±17	100	±18
7	—	37.3	36.8–37.6	97	±20	91	±14	37.2	36.8–37.6	83	±26	84	±14

Materials and methods

The study was made on series of 22 patients who had been admitted for the so-called endogenous type of bronchial asthma, which had set in many years previously. The patients were divided into two groups.

1. A control group of 11 patients (7 men and 4 women) who had received no corticosteroid therapy and were from 37 to 62 years of age (mean age, 57 years).

2. A steroid-treated group of 11 patients (8 men and 3 women) who daily for 18 to 62 months (mean period, 35 months) had received 5 to 10 mg of prednisone or equivalent doses of other corticosteroids and were from 35 to 74 years of age (mean age, 57 years).

These patients' excretion of urinary steroid metabolites was estimated according to Korymberski et al. (14) and used for determining the 17-ketogenic steroid and 17 ketosteroid excretions. In addition the 17 OHCS content of blood was estimated by the method of Eik-Nes (6).

These estimations were made when the patients were afebrile and were repeated after fever had been induced by an intravenous injection of from 0.6 to 1.2 ml, depending on body weight, of a pyrogenic vaccine containing 100 million *Bacillus alkaligenes* per ml. Fever commences 4 to 5 hours and attains maximum of some 39° C. 6 to 8 hours after such an injection. Mean rectal afternoon temperatures are given in table I.

The patients were examined during a period when they were essentially free from asthmatic symptoms, had no complicating diseases and were afebrile. Those patients who had undergone steroid therapy had discontinued such therapy 72 hours before the tests began. Then the 24-hour urinary steroid excretion was determined on each of the next three days. At 8 o'clock a.m. on the 4th day the patient was given an injection of pyrogenic vaccine. The 24-hour urinary steroid excretion was determined for that day as well. Concomitantly the 17-OHCS content of blood was estimated t 8 a.m., 12 noon, 3 p.m. and 7 p.m. on the 4th day and also at 7 a.m. on the 5th day. Lastly 24-hour urinary steroid excretions were determined for the 5th, 6th and 7th days.

that not even ACTH administration provides any assurance of permanent restoration of adrenocortical function (5)

These and related problems seem to have aroused considerably controversy among early workers. A likely explanation for the divergent opinions is that the authors treated different diseases and/or administered dissimilar corticosteroid doses.

Bronchial asthma seems to occupy a position of its own among the many diseases that have been treated with corticosteroids, the reason being that it responds well to long term administration of comparatively small steroid doses — usually 5 to 10 mg is an adequate daily dose of prednisone or an equivalent steroid drug Whereas it has not been fully established whether such small doses actually are capable of inhibiting pituitary adrenocortical activity a previous investigation (3) disclosed that only very rarely can any signs of such inhibition be demonstrated Thus the basal urinary 17 ketogenic steroid and 17 ketosteroid excretions were normal in all patients in a series of 144 who had been treated with corticosteroids continuously for an average period of 2 years. Moreover after an 8-hour intravenous ACTH infusion the corticosteroid excretion remained normal even if slightly delayed in a few cases. None of the patients concerned had received any intercurrent ACTH injections as a prophylaxis against inhibited adrenocortical function The investigation just mentioned showed also that patients who had undergone long term corticosteroid therapy responded normally to acute situations of stress. For example infections with high fever would be overcome without the pituitary adrenocortical system showing signs of failure. Only a single case of "withdrawal syndrome" could be diagnosed and, being deemed particularly interesting, it will now be described briefly

Case report

A woman of 50 with asthma since 1941 who had been taking an average of 7.5 mg prednisone daily since 1956. X-ray evidence of a steroid-induced peptic ulcer was present on two occasions. The patient was admitted to the hospital Oct. 4th 1960 The blood pressure was 150/90 and steroid therapy was withdrawn. Two days later the patient complained of fatigue, vertigo, malaise and vomiting The blood pressure had dropped to 85/55. The 24-hour urinary excretions of 17 ketogenic steroids and 17 ketosteroids on Oct. 6th were 0.9 mg and 0.8 mg respectively The corresponding figures for Oct. 8th were 9.6 and 2.3 mg An intravenous ACTH test showed subnormal values, with 24-hour urinary excretion of 21 mg 17-ketogenic steroids and 1.9 mg 17-ketosteroids. The patient's symptoms vanished a few days after resumption of steroid therapy

Surgical interventions and febrile conditions are the commonest states of stress to which patients are exposed Frankson's et al. (7) investigations have disclosed that operations are attended by a sharply increased level of blood cortisone in the majority of patients. It is known too that in most cases the same occurs in conjunction with febrile conditions. Furthermore it can be shown that the urinary steroid excretion rises under similar conditions (8) For the purposes of the present investigation therefore, we decided to estimate the adrenocortical function from hormone assays before, during and after induction of fever in patients with bronchial asthma who had undergone long term corticosteroid therapy and also in similar patients who had received no such treatment

able I. Urinary excretion of 17-ketogenic and 17-ketosteroids in patients with bronchial asthma, before ring and after induced fever

y	Treatment	Patients not previously treated with corticosteroids						Patients previously treated with corticosteroids					
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				17-ketogen		17-keto				17-ketogen		17-keto	
		Mean	Range	Mean	S. E. of mean	Mean	S. E. of mean	Mean	Range	Mean	S. E. of mean	Mean	S. E. of mean
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Table II 17-OHCS concentration in blood at different times of day in normals and in pyrogen-treated asthmatics with and without long-term corticosteroid therapy

Time of day	Normals	Pyrogen-treated patients with bronchial asthma	
		Not steroid-treated	Steroid-treated
8 a. m.	13.6 (8.8–18.4)	14.4 (2.8–21.3)	12.6 (6.0–20.9)
12 noon	11.0 (6.6–15.0)	13.4 (6.6–22.2)	12.7 (5.3–21.9)
3 p. m.	9.0 (4.8–12.2)	16.9 (10.2–26.5)	16.7 (11.2–25.3)
7 p. m.	6.2 (2.2– 8.4)	11.7 (1.8–24.9)	10.6 (3.8–24.2)
7 a. m.	14.4 (9.4–19.2)	12.8 (5.6–22.5)	14.8 (8.7–?1.2)

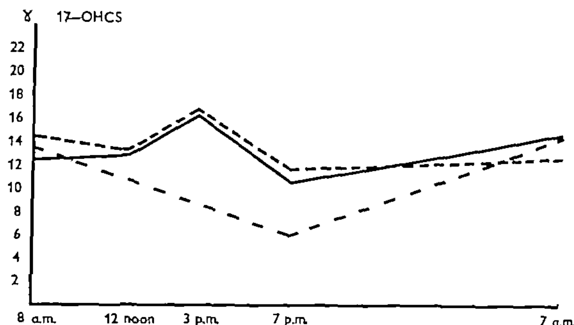


Fig. 1 17-OHCS content of blood at various times on the day of artificial fever induction.
——— Mean for controls
——— Mean for steroid-treated patients
— — Mean for normal diurnal rhythm

Results

The results are summarized in tables I and II and plotted graphically in fig 1

Table I shows that pyretotherapy induced a fever of about 39 C in both groups. While the rectal temperature remained slightly above normal next after noon, it fell to normal levels on the day after that. The mean 24-hour urinary excretions of both 17 ketogenic steroids and 17 ketosteroids rose during the febrile period. Owing to great variations from patient to patient the standard errors of the mean are very high.

The excretion of 17 ketogenic steroids rose in 10 and that of 17 ketosteroids in 9 *of the* 11 *controls.* Similarly among the 11 patients in the steroid treated group, 10 showed a rise of 17 ketogenic steroid

as well as of 17 ketosteroid excretion. The mean increases were tested for significance by the sign-test method (11) the result being that the increases in 17-ketogenic steroids as well as in 17 ketosteroids during pyretotherapy were significant (P < 0.05) in both groups (cf. 8) No significant differences can be demonstrated between the groups.

Table II shows that the 17-OHCS content of blood was increased by the fever during a period of an hour or two centered on the time for the fever peak. No significant differences appear between the steroid-treated group and the control group (fig 1)

Discussion

In patients with bronchial asthma who have not been treated with corticosteroids, fever increases both the 17-OHCS content of blood and the urinary excretions of 17 ketogenic steroids and 17 ketosteroids, as in part was made apparent by our previous report. Such increases are dependent on unimpaired adrenocortical function Hence both the synthesis and the release of ACTH also must be impaired. Against such a background these experiments confirm the opinion expressed previously that impaired function of the pituitary-adrenocortical system has not been demonstrated in asthmatics.

In our opinion the results suggest that the small steroid doses adopted, even if administered continuously over a period of many years, do not give rise to any functional impairment of the pituitary adrenocortical system that can be detected by available methods. At any rate they indicate that the functional reserve capacity remains sufficient for an adequate response to reasonable stress situations The instances of complications reportedly ascribed to adrenal deficiency secondary to steroid therapy would seem to constitute occasional and isolated cases, perhaps due to high doses.

Since many years this Department has had a large clientele of asthmatic patients who are undergoing continuous steroid therapy In such cases the steroid medication has often been withdrawn abruptly for example in connection with follow-up examinations, or to check whether the long-term steroid treatment must be continued. Stress situations of various types have consequently been common. Nevertheless with the sole exception described in the foregoing we have never seen a conclusive case of so-called withdrawal syndrome. Accordingly no supporting ACTH therapy either regular or terminal, has been used. Since we cannot at present tell how these patients would respond to major stress situations, such as surgical interventions, we administer supplementary cortisone doses on such occasions.

The present investigation has shown that a series of asthmatic patients who had received comparatively small daily corticosteroid doses for an average period of 3 years did not differ from a similar group of patients who had not received corticosteroid therapy in respect of their ability to increase their endogenous production of corticosteroids when exposed to the stress of fever

The conclusions given above do not apply to cases where large corticosteroid doses are given.

Summary

Twenty two patients with bronchial asthma of the so-called endogenous type have been examined Eleven of them had previously been treated for an average

period of 3 years with corticosteroids in *small doses (corresponding to a daily* prednisone dose of 5 to 10 mg) the 11 others had received no such treatment and were used as controls.

The steroid treatment of the first group was withdrawn 3 days before the investigation began. The 24-hour urinary 17 ketogenic steroid and 17 ketosteroid excretions were then assayed in both groups on 7 consecutive days. On the 4th day artificial fever (about 39 C) was induced in all patients and the 17-OHCS content of their blood was determined

The results disclosed that artificial fever induced a significant increase of both the 17-OHCS content of blood and the urinary steroid excretions in the two groups. This suggests that patients under going prolonged treatment with small to *moderate steroid doses* possess adequate pituitary adrenocortical reserve to react normally to common stress situations.

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Effect of a Thyroxine Analogue (Triiodothyropropionic Acid) on the Calcium-phosphorus Metabolism

By

S. Hardemann, Th. Friis and N. I. Nissen

A number of recent publications have dealt with the favourable effect of various thyroxine analogues upon an elevated serum cholesterol, which they reduce without correspondingly raising the basal metabolic rate (7—16, 21—31).

It has been demonstrated, among other features, that triiodothyropropionic acid (triprop) in doses of 2—6 mg daily is capable of decreasing hypercholesterolaemia by an average of 26 % associated with an average increase in the B. M. R. of only 6 % and without inducing any appreciable thyrotoxic symptoms. In similar studies using triiodothyronine (T_3) the patients with hypercholesterolaemia have responded by a significantly less marked decrease in cholesterol (6 %) while the B. M. R. increased equally on a dosage of 40—60 μg daily (13).

It has often been stated that hyperthyroidism is associated with an increased loss of calcium with the urine and faeces (2, 8, 18, 19, 33). In a previous paper one of the authors (12) demonstrated that in addition to hypercalcuria there is often a reduced tubular reabsorption of phosphorus in the kidneys just as in hyperparathyroidism. This accords with the findings of Bessel et al. (4), Flach et al. (9), Hetzel et al. (15) and Welby et al. (34) that T_3 may induce an increased phosphaturia in human subjects as well as dogs.

We, therefore, felt prompted to investigate whether triprop in the dosage stated exerted a similar effect upon the excretion of calcium and phosphorus.

Material and methods

The material comprised the following groups of euthyroid patients some of whom had a normal and others an elevated serum cholesterol. 1) 10 treated for 11 days with 4—6 mg triprop, 2) 8 treated for 6—12 months with 2—4 mg triprop, 3) 9 treated for 11 days with 40—80 μg T_3, and 4) 5 treated for 1—3 months with 40—60 μg T_3.

All the patients had determination of the 24-hour urinary output of calcium on an ordinary diet and some of them on a low-

Submitted for publication December 20, 1962

period of 3 years with corticosteroids in small doses (corresponding to a daily prednisone dose of 5 to 10 mg) the 11 others had received no such treatment and were used as controls.

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Effect of a Thyroxine Analogue (Triiodothyropropionic Acid) on the Calcium phosphorus Metabolism

By

S. Hahnemann Th. Friis and N I Nissen

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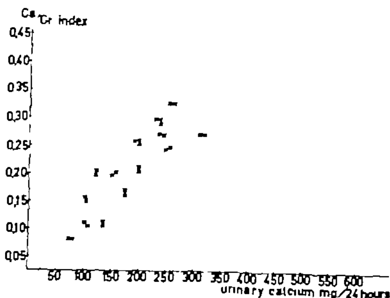


Fig 1 Relation between calcium-creatinine index and 24-hour urinary output of calcium in untreated patients on an ordinary diet.

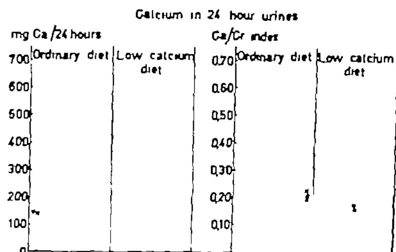


Fig 2. Scatter diagram comparing the calcium creatinine index and 24-hour urinary calcium on an ordinary diet and on a low-calcium standard diet.

calcium standard diet (= about 150 mg Ca daily) for 3 successive days (EDTA method (22)) determination of the tubular reabsorption of phosphorus on an ordinary diet (12) the phosphorus being determined by the method of Muller (26) and creatinine by the method of Bonnes and Tausky (6) determination of the B.M.R. protein-bound iodine in the serum (3) triiodothyronine uptake (T_3 uptake) by the red cells (11) and serum calcium (EDTA method) as well as alkaline phosphatases (17) Furthermore, we determined the calcium creatinine index in the 24-hour urine (28) stated as the ratio between the calcium and creatinine concentration, both measured as mg/100 ml In normal subjects this ratio is stated to be below 0.28 (28) All the investigations were performed before as well as during the treatment. Lastly the excretion of calcium in the faeces and urine before and during treatment with 4—8 mg triprop was investigated in 5 patients on a low-calcium standard diet (= about 150 mg Ca in the 24 hours)

Results

First, a comparison of the calcium creatinine index in the urine with the daily urinary output of calcium

Fig 1 shows the relation between the calcium-creatinine index and 24-hour urinary output of calcium on an ordinary

Table I. Alterations in calcium-phosphorus output, serum cholesterol, and basal metabolic rate in 10 euthyroid patients treated with 4—6 mg triiodothyropropionic acid for 11 days

Case No.	Dosage (mg)	Urinary Ca (mg/24 hrs)		Urinary Ca (% of init. value)		Ca/Cr index		Ca/Cr index (% of init. value)		TRP* (% of init. value)	Serum cholesterol		BMR (%)
		Ord. diet	St. diet	Ord. diet	St. diet	Ord. diet	St. diet	Ord. diet	St. diet		mg/100 ml	% of init. value	
1	6	+161	—	+163	—	+0.124	—	+77	—	− 2.8	− 72	−22	+12
2	6	+ 82	—	+ 26	—	+0.025	—	+ 6	—	− 1.6	− 17	− 7	+15
3	6	+113	—	+ 23	—	+0.193	—	+56	—	− 4.0	−109	−27	+14
4	6	+123	—	+ 42	—	+0.269	—	+90	—	+ 1.7	− 95	−33	+22
5	6	+ 52	—	+ 15	—	+0.054	—	+19	—	+ 3.9	− 36	−11	+23
6	6	+ 33	—	+ 12	—	+0.070	—	+32	—	+ 1.2	− 60	−16	+12
7	6	− 7	—	− 2	—	+0.054	—	+22	—	− 4.9	− 5	− 2	−10
8	4	—	+ 13	—	+11	—	+0.021	—	+ 19	−17.1	− 45	−27	—
9	4	—	+ 91	—	+40	—	+0.179	—	+104	− 1.7	− 14	− 9	—
10	4	+156	+157	− 59	+73	+0.073	+0.114	+42	+ 85	+ 3.1	—	—	+ 2
Mean		+ 82.0		+ 40		+ 0.108		+ 48.9		− 2.2	− 50	−17.1	+11.5
Border line ranges		−7—+161		−2 +163		+0.021—+0.269		+6—+104		+ 3.9— −17.1	5— −109	− 2 −33	−10— +23

TRP = Tubular reabsorption of phosphorus.

diet in 31 untreated patients without any signs of a disturbance of the calcium-phosphorus metabolism. A total of 95 analyses were performed. There is a distinct linear relation, but with a tendency to an asymptomatic course, a larger number of patients having an increased 24-hour urinary output of calcium (> 300 mg/24 hours) (10 patients, 28 analyses) than a calcium-creatinine index exceeding 0.30 (7 patients, 22 analyses).

Fig. 2 gives the 24-hour urinary output of calcium on an ordinary diet and on a low-calcium standard diet in 16 untreated patients (46 analyses) as well as the calcium-creatinine index in 13 untreated patients (37 analyses). The three patients in whom the calcium creatinine index was not measured showed a 24-hour urinary output of calcium below 300 mg. While 10 analyses showed an increased 24-hour urinary output of calcium on an ordinary diet, 6 had an increased calcium creatinine index. On the low calcium standard diet 3—4 analyses showed values over 300 mg/24 hours and 0.30 respectively. In other words, in individuals having no abnormalities of calcium-phosphorus metabolism there seems to be, as emphasized by Nordin (28) a tendency for the calcium-creatinine index to show fewer elevated values of the calcium output on an ordinary diet than suggested by the 24-hour urinary output of calcium.

Table I lists the results of the treatment of group I (treated for 11 days with 4—6 mg triprop). The alterations in urinary calcium and in the calcium creatinine

Table II Alterations in calcium-phosphorus output, serum cholesterol and basal metabolic rate in 8 patients with hypercholesterolaemia treated for from 6 to 12 months with triiodothyropropionic acid 2—4 mg daily

Case no.	Dosage (mg)	Urinary Ca (mg/24 hrs)		Urinary Ca (% of init. value)		Ca/Cr index		Ca/Cr index (% of init. value)		TRP (% of init. value)	Serum cholesterol		BMR (%)
		Ord. diet	St. diet	Ord. diet	St diet	Ord. diet	St. diet	Ord. diet	St diet		mg/100 ml	% of init. value	
1	4	+182	–	+55	–	+0.122	–	+ 45	–	+6.0	−117	−23	+18
2	4	–	+ 91	–	+20	–	–	–	–	−4 1	−110	−19	+ 8
3	4	+110	–	+38	–	–	–	–	–	+5.2	− 92	−17	+ 7
4	4	+101	+179	+61	+97	+0 182	+0 199	+102	+119	−6.2	−105	−25	+13
5	4	+ 69	+115	+27	+58	+0 106	+0.135	+ 41	+ 58	−1.9	−245	−53	+ 2
6	2	+ 49	–	+33	–	+0.049	–	+ 41	–	+2.2	− 17	− 5	+24
7	2	− 9	+ 4	− 6	+ 2	+0.009	+0.024	+ 4	+ 11	−3 7	− 13	− 3	+12
8	2	− 8	+ 31	− 3	+13	−0.026	+0.043	− 9	+ 16	−0.6	+ 25	+ 7	+ 5
Mean		+ 71	+ 84	+29	+38	+0.074	+0.100	+ 37	+ 51	−0.4	− 82	−17	+11
Border line range		−9– +182	+4– +179	−6– +61	+2– +97	−0.026– +0 182	+0.024– +0.199	−9– +102	+11– +119	−6.2– +6.0	+25– −245	+7– −53	+2 +24

TRP = Tubular reabsorption of phosphorus

index will be designated as the difference between the mean values of the deter mination on 3 successive 24-hour urines. In 4 of the 10 subjects the initial values of the 24-hour urinary output of calcium averaged *more than 300 mg/24* hours on an ordinary diet. In a further 4 patients the 24-hour urinary output of calcium rose to more than 300 mg/24 hours In 9 out of 10 the excess excretion of calcium ranged from 13 to 161 mg/24 hours (11—163 % of the initial values) The increase showed no relation to the initial values. In one case (No 7) there was no increase. In cases 1—7 treated with 6 mg triprop daily there was an average increase of 79 9 mg/24 hours (40 1 % of the initial value) In cases 8—10 cured on 4 mg daily there was an average in crease of 87 mg/24 hours = 39 % of the initial value.

The calcium-creatinine index increased by 0 021—0 269 (6—104 % of the initial values) In cases 1—7 the average increase amounted to 0 113 (43 7 %) in cases 8—10 to 0 098 (62 %)

One patient (case 8) showed a significant decrease in the tubular reabsorption of phosphorus in per cent of the amount filtered. This was accompanied by an in crease in serum phosphorus. The other patients showed no definite changes.

Serum cholesterol showed a decrease from 5 to 109 mg/100 ml (2—33 of the initial values without *any definite relation* to the latter) In cases 1—7 there was an average *fall of 56 3 mg/100 ml* (15 9 %) In this respect too the alterations are calculated as the difference between the mean values of 3 successive determina tions. Two patients showed elevated initial values.

Table III. Alterations in calcium-phosphorus output, serum cholesterol and basal metabolic rate in 9 euthyroid patients treated for 11 days with 1-triiodothyronine 40 60 and 80 µg daily

Case No.	Dosage (µg)	Urinary Ca (mg/24 hrs)		Urinary Ca (% of init. value)		Ca/Cr index		Ca/Cr index (% of init. value)		TRP¹ (% of init. value)	Serum cholesterol		BMR (%)
		Ord. diet	St. diet	Ord. diet	St. diet	Ord. diet	St. diet	Ord. diet	St. diet		mg/100 ml	% of init. value	
1	60	− 32	−	−26	−	+0.096	−	+ 87	−	− 5.2	+ 72	+50	+ 3
2	60	+ 89	−	+84	−	+0.026	−	+ 41	−	− 5.7	− 85	−22	+ 4
3	60	+195	−	+87	−	+0.197	−	+ 80	−	+ 1.3	− 35	−13	+ 9
4	60	+100	−	+37	−	+0.172	−	+ 46	−	+10.5	− 55	−18	+ 4
5	60	+ 57	−	+20	−	+0.072	−	+ 31	−	+ 4.1	− 77	−15	+11
6	80	+102	+150	+54	+73	+0.248	+0.393	+102	+163	−11.6	− 81	−33	+ 3
7	80	+123	+ 61	+89	+37	+0.179	−	+ 89	−	− 2.6	− 1	−14	+22
8	40	+ 18	−	+21	−	+0.037	−	+ 38	−	−15.6	+117	+36	+ 6
9	40	+144	− 28	+36	− 6	−0.090	+0.039	− 17	+ 10	− 3.6	− 27	− 7	+ 3
Mean		+ 70.1		+ 40.6		+ 0 119		+ 60.9		− 3.2	− 21.1	− 4	+ 7 4
Border line range		−32—+195		−26—+87		−0.023—+0.321		−3—+153		+10.5—−15 6	+117—− 85	+50—−33	+3—+22

TRP = Tubular reabsorption of phosphorus.

The B. M. R. changed from — 10 to + 23 % (m = 12.6 %) in cases 1—7

The PBI rose in all cases to more than 10.0 µg/100 ml, while the T uptake by the red cells did not undergo significant alterations. There were no definite alterations in serum calcium or alkaline phosphatases.

Table II sets out the alterations in some of the laboratory data in group 2 treated for 1/2—1 year with 2—4 mg triprop.

In 2 out of the 8 patients the initial values of urinary calcium exceeded 300 mg/24 hours. In a further 2 patients the 24-hour urinary output of calcium rose to more than 300 mg/24 hours. An increase occurred in 6, calculated as described above 1) in 5 patients treated with 4 mg daily from 91 to 182 mg/24 hours (20—79 % of the initial values, m = 47 %) 2) in 3 patients treated with 2 mg daily between — 3 and + 49 mg/24 hours (m = 19 mg/24 hours) (—2 to + 25 % of the initial values, m = + 9 %) (mean values for ordinary and standard diet are used)

The calcium-creatinine index showed the following alterations 1) Patients treated with 4 mg daily 0 121—0 191 (m = 0.149 = + 68 % of the initial value) 2) Patients treated with 2 mg daily + 0 009 to + 0.049 m = 0.028 (4—41 % of the initial values = + 18 %) (mean values for ordinary and standard diet are used)

There were no significant changes in TRP or serum phosphorus.

The fact that this group of patients also showed an increased urinary output of calcium indicates that this increase was not a transitory phenomenon.

Serum cholesterol showed the following

Table IV Alterations in calcium-phosphorus output serum cholesterol, and basal metabolic rate in 5 euthyroid patients treated with l triiodothyronine 40—60 µg daily for 1—3 months

Case no.	Dosage (µg)	Urinary Ca (mg/24 hrs)		Urinary Ca (% of init. value)		Ca/Cr index		Ca/Cr index (% of init. value)		TRP[1] (% of init. value)	Serum cholesterol		BMR (%)
		Ord. diet	St. diet	Ord. diet	St. diet	Ord. diet	St. diet	Ord. diet	St. diet		mg/100 ml	% of init. value	
1	40	+32	− 1	+17	−0.5	+0 084	+0 032	+34	+13	+2.3	—	—	—
2	40	+21	—	+25	—	+0.042	—	+41	—	−7 7	+110	+34	+1
3	40	+11	− 6	+ 3	− 1	−0.034	−0.032	− 7	− 8	+3.3	− 20	− 5	+2
4	40	+30	+86	+12	+43	+0.049	+0.078	+19	+33	+3.3	−130	−28	−4
5	60	+35	+68	+15	+31	+0 069	+0 094	+22	+23	+8.5	− 58	−13	+1
Mean		+26	+37	+14	+18	+0.042	+0.027	+22	+15	+1.9	− 12	−2.4	0
Border line range		+11– +35	−6– +86	+3– +25	−1– +43	−0.034– +0 084	−0.032– +0.094	−7– +41	−8– +33	−7.7– +8.5	+110– −130	+34– −28	−4– +2

TRP = Tubular reabsorption of phosphorus.

alterations 1) In patients treated with 4 mg daily there was a decrease of between 92 and 245 mg/100 ml m = 134 mg/100 ml (19—56 % of the initial values m = 25 %) 2) Patients treated with 2 mg showed alterations between + 25 and — 17 mg/100 ml, m ≐ — 2 mg/100 ml (+ 5 to — 5 % of the initial values, m = — 1 %) All had shown *elevated initial values.*

In the B.M.R there were the following alterations 1) Patients treated with 4 mg daily from + 2 to + 18 % m = 10 % 2) Patients treated with 2 mg daily from + 5 to + 24 % m = + 14 %

In all cases there was an increase in PBI to more than 10 0 µg/100 ml while there were no definite alterations in the T_3 uptake by the red cells in serum calcium, or in alkaline phosphatases.

Table III shows the results of the short term experiments using T_3 In these cases too the urinary output of calcium rose without relation to the initial values which had been more than 300 mg/24 hours in cases 1 and 9 In further 4 patients the 24-hour output of calcium rose to more than 300 mg/24 hours. In cases 1—5 treated with 60 µg/24 hours, there was an average increase of 81 mg/24 hours or + 40 4 % of the initial values. There was an average alteration in the calcium creatinine index of + 0 111 or + 59 % of the initial values.

Cases 1—5 showed an average decrease in serum cholesterol of 36 mg/100 ml or — 3 6 % of the initial values which had been elevated in 3

There was a significant fall in TRP in cases 6 and 8, accompanied by an increase in serum phosphorus. The other patients showed no definite alterations.

In these 5 cases the B.M.R. rose by an average of 6 %

There was a slight tendency to a fall in PBI without a definite alteration in the

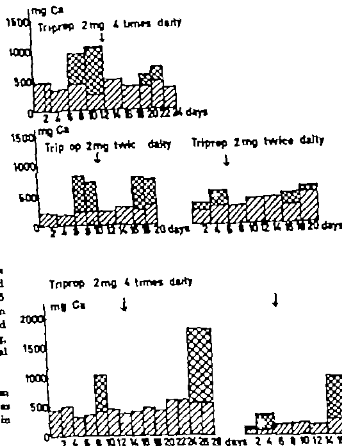


Fig. 3. Excretion of calcium in the urine and faeces before and during triprop medication in 3 patients on low-calcium standard diet. Single-hatched area = urinary calcium in mg, double-hatched area = faecal calcium in mg
Abcissa: Days.
Ordinate: Excretion of calcium in mg/24 hours calculated as the average of the excretion in 2—3 days.

T uptake by the red cells. No definite alterations were found in serum calcium r alkaline phosphatases.

Table IV gives the esults in group 4 reated in 1—3 months with 40—60 μg T_3. Cases 1 2, and 3 are identical with cases 6 8 and 9 in group 3 In one out of five patients the initial values of urinary calcium exceeded 300 mg/24 hours. In no further patients the 24-hours urinary out put of calcium rose to more than 300 mg/ 24 hours.

Cases 1—4 treated with 40 μg daily showed a slight increase in urinary calcium m = 24 mg/24 hours or + 16 % of the initial values. The investigations on a low-calcium standard diet showed a less marked increase. Case 5 treated with 60 μg/24 hours, exhibited a somewhat greater increase on both diets.

In cases 1—4 there was an average increase in the calcium-creatinine index of 0.035 or 22 % of the initial values on an ordinary diet. On the standard diet the nvestigations showed similar alterations. Case 5 showed a distinct increase on both diets.

There were no significant alterations in TRP or serum phosphorus. The alter ations occurring in cases 6 and 8 (table III) after a short time of treatment subsided with continued therapy in case 6

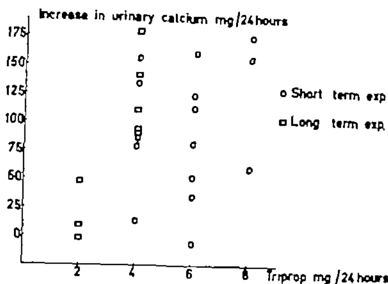


Fig 4 Relation between alteration in urinary excretion of calcium in mg/24 hours and dose of triprop mg/24 hours.

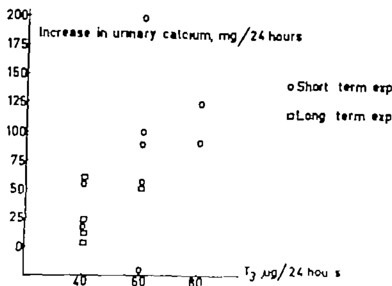


Fig 5. Relation between alteration in urinary output of calcium in mg/24 hours and dose of T_3, µg/24 hours.

after the dosage had been reduced from 80 to 40 µg daily

Serum cholesterol fell in 3 cases and *increased in one. In 3 there had been* elevated initial values.

The B.M.R. increased by 1—2 %

In PBI there was a *marked decrease to* values around 1 µg/100 ml without definite changes in the T_3 uptake by the red cells. There were no *definite alterations in serum-calcium or alkaline phos*phatases.

It may be mentioned that no group *showed significant changes in serum* or urinary creatinine.

Fig 3 illustrates the effect of triprop *in a daily dose of 4—8 mg upon* the urinary and faecal output of calcium on a low-calcium standard diet in 5 patients. *Of course* these findings must be assessed

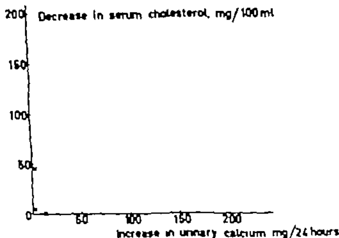


Fig. 6. Relation between increase in urinary output of calcium and decrease in serum cholesterol on triprop medication.

with a certain reserve, partly because of the individual differences in the onset of adaptation to a low calcium intake demonstrated by Malm (23) and partly because of the short experimental periods.

In 2 instances there was a tendency to an increase in faecal calcium, while in the other cases there were no definite changes. Two of the latter patients were treated with 4 mg daily the others with 8 mg daily In 4 of the patients there was an increase in the urinary output of calcium.

Discussion

The present investigations revealed that triprop, in doses of 4—8 mg daily entailed a significant increase in the urinary output of calcium, while there was no consistent effect upon the faecal excretion of calcium. However there does not appear to be an increased absorption from the intestinal tract to compensate for the increased urinary excretion.

Rawson et al. (32) found an increase in the urinary as well as faecal output of calcium in one patient with myxoedema who received triprop in a single intravenous dose of 10 mg On the other hand, Benua et al. (5) using the same experimental procedure on the same type of patient, found no effect upon the calcium excretion. To our knowledge there have not been any previous studies on the effect of long-term treatment with triprop.

Only one out of 17 patients showed a reduction in TRP associated with an increased urinary excretion of phosphorus during triprop therapy This patient showed at the same time a distinct increase in serum phosphorus. Rawson et. al (32) as well as Benua et al.s (5) patients showed an increase in urinary phosphorus as well as in serum phosphorus.

T in doses of 60—80 µg daily induced n increase in the urinary output of calcium of an order of magnitude corresponding to the effect of 4—6 mg triprop. This is apparent from figs 4 and 5 which show the relation between the dose of triprop and T and the urinary output of calcium. Obviously there are marked individual differences, but both 2 mg triprop and 40 µg T caused only a slight increase.

Only 2 out of 11 patients exhibited an increase in the urinary excretion of phos-

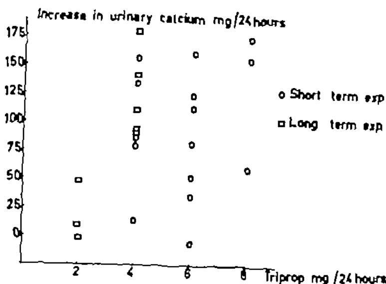


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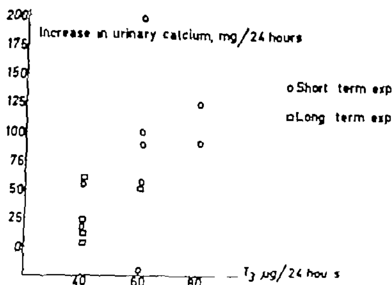


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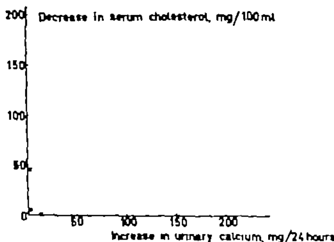


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Only 2 out of 11 patients exhibited an increase in the urinary excretion of phos-

phorus during T_3 therapy which in these cases too was accompanied by an increase in serum phosphorus. As already mentioned these alterations, observed in both cases in the short term treated group subsided on continued medication.

These results contrast with the findings from studies on the immediate effect of T_3 administered intravenously in single doses to normal subjects of patients with myxoedema (4 15 31) These previous studies showed fairly consistently a distinct increase in the urinary output of phosphorus, but no definite effect upon urinary calcium. On the other hand our results correspond with the findings in thyrotoxicoses (2 12 19 33) and during daily oral administration of thyroid (2 33) there being in normal subjects as myxoedematous patients an increase in the urinary as well as faecal excretion of calcium and phosphorus

The present investigations revealed a pronounced decrease in serum cholesterol on triprop in daily doses of from 4 mg and upwards, accompanied by a rather slight increase in the B.M.R. as also reported previously It is apparent from the results, however that the thresholds must lie very close together for an effect upon the urinary excretion of calcium and that upon serum cholesterol. And indeed fig 6 shows that apparently there is a relation between an increase in urinary calcium and a decrease in serum cholesterol.

Thus, if the hypocholesterolaemic effect of triprop is to be utilized administering the drug in daily doses of 4 mg and over a certain loss of calcium must be expected. If this loss of calcium persists for some length of time it may perhaps lead to the development of osteoporosis or osteitis fibrosa as described in cases of hyperthyroidism (1 10 27) Whether this risk may be reduced by an increased calcium phosphorus intake cannot be decided as yet but *some investigations* on hyperthyroidism indicate that it can (18, 30)

The fairly high urinary excretion of calcium observed in some cases involves a theoretical possibility of the formation of renal calculi. Since, however renal calculi do *not* occur in thyrotoxicosis (25) in which the urinary output of calcium may exceed 1 000 mg/24 hours (2) it would appear reasonable to put forth the idea that in thyrotoxicosis and in tolerance tests of the type described here there may be a question of an increased solubility of calcium in the urine due e.g to an increased excretion of citric acid (14) or amino acids (24) or to other factors (29)

As far as the effect of triprop and T_3 upon PBI is concerned the increase during administration of triprop is presumably due to an action of iodine while the decrease observed after lengthy administration of T must be interpreted as the result of an inhibition of the thyroid gland (20) At the 100 times lower dosage of T_3 the latter effect overshadows the iodine effect observed on administration of triprop

The comparison of the calcium-creatinine index and the 24-hour urinary output of calcium to assess the urinary output of calcium in normal subjects, forms the basis of further studies, partly on larger normal series and partly on patients having disturbances of the calcium phosphorus metabolism. Should the calcium-creatinine index give more consistent information regarding the urinary output of calcium and show less dispersion within the normal range as indicated by Nordin s studies (28) as well as ours, it would afford an applicable clinical test which, apart from the advantages already mentioned would save the work and

problems of an accurate collection of 24-hour urines, since according to Nordin this index may be determined on an ordinary sample of urine. Furthermore, Nordin states that the calcium-creatinine index appears to be less susceptible to alterations in the calcium intake. This is at any rate not contradicted by our findings, and it involves the possibility of performing the test on the patient while he is on his normal diet.

As is apparent from the tables, there occurred during the tolerance tests alterations in the calcium-creatinine index almost parallel to the alterations in the 24-hour urinary output of calcium.

If triprop is to be used in the treatment of hypercholesterolaemia, the effect upon the calcium metabolism makes it necessary to tighten up the indications for the treatment of hypercholesterolaemia, and the urinary output of calcium must be closely observed (e g by Sulkowitz reagent) In the event of an increase in urinary calcium, the dosage should be reduced and the diet supplemented with extra calcium and phosphorus.

Summary

Investigations with the particular aim of elucidating the effect of triiodothyropropionic acid and l triiodothyronine upon the metabolism of calcium and phosphorus were carried out on 16 normocholesterolaemic and 16 hypercholesterolaemic euthyroid patients.

The results showed a significant increase in the urinary output of calcium during administration of the drugs in therapeutic doses. This effect must be interpreted as a by no means harmless side effect of a possible therapeutic use of triiodothyropropionic acid in a daily dosage of 4 mg and over in the long-term treatment of hypercholesterolaemia.

Acknowledgement

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Lipid Metabolism and Trauma

II. Studies on the Effect of Nicotinic Acid on Norepinephrine Induced Fatty Liver

By

Lars A. Carlson and Sten-Otto Liljedahl

In a previous study we found that 24 hours after surgical trauma in the dog the liver glycerides increased (2). As shown earlier (12) the plasma free fatty acids (FFA) were also found to increase after trauma. Furthermore, the increase in liver glycerides was directly proportional to the increase of FFA (2). Similar findings, i. e. increased levels of FFA and increased liver glycerides, are seen after infusion of norepinephrine (5). It was also found that pretreatment of dogs before trauma with guanethidine, a peripheral sympathetic inhibitor prevented the increase in FFA as well as in liver glycerides after trauma (2).

The effect of catecholamines on lipids in the blood and different organs has been the subject of much interest for many years. Thus it was demonstrated in 1937 that epinephrine given to rats rapidly caused a fatty liver (10). The same has been shown to occur after administration of norepinephrine (1). The recent progress in our understanding of lipid metabolism and fatty acid transport has facilitated the understanding of the events leading to fatty infiltration of different organs. Thus the free fatty acid (FFA) fraction of the plasma lipids has been shown to have an extremely rapid turnover (7) to be mobilized from the adipose tissue to other organs (7) and to be under control of the sympathetic nervous system (8). It has been shown that about one third of the circulating FFA are taken up by the liver and are there rapidly esterified (9 11). Furthermore the uptake of FFA by the liver is roughly proportional to the concentration (6). Thus, an increased FFA concentration, induced e. g. by catecholamines, might lead to an accumulation of lipids in the liver if the capacity for disposal of the lipids, e. g. via oxidation and secretion of lipoproteins, is exceeded. A recent study of fatty liver indicated that the fatty

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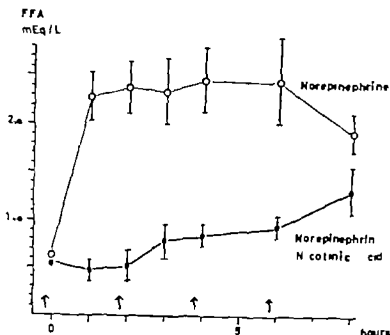


Fig 1 Arterial plasma FFA concentration during constant infusion of norepinephrine from 0 hours (0.5 μg/kg/min.) to anesthetized dogs. One group was in addition given nicotinic acid as indicated by the arrows and described in the text. The vertical bars indicate the standard error of the mean.

acids of the liver lipids after nor epinephrine infusions were derived from FFA and not from *de novo* synthesis in the liver (5) Although the evidence so far is impressive that the catecholamine induced fatty infiltration of the liver is due to the increased flux of FFA through plasma other mechanisms might also be involved It has thus not been ruled out that the pressor effect or other effects of norepinephrine e g influences the capacity of the liver to handle the load of FFA in such a way that lipids ac cumulate. In order to elucidate this problem further we have used a specific metabolic sympathicolytic agent, viz. nicotinic acid during continuous infusion of norepinephrine and studied the effect on FFA and the liver lipids Nicotinic acid has recently been shown to block the increase of FFA caused by catechol amines without affecting the pressor response (3) Furthermore nicotinic acid blocks the direct stimulating effect of catecholamines on the release of FFA and glycerol from adipose tissue *in vitro* (4)

Material and methods

Animals and experimental procedure

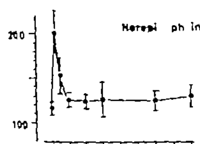
Six adult mongrel dogs, 15—20 kg, were used after fasting for about 24 hours. The dogs were anesthetized with 30 mg/kg body weight of Nembutal®

After induction of anesthesia a liver biopsy was obtained through a mid-line incision. The liver was sutured and the abdominal wall closed. Then l norepinephrine in saline was given intravenously at a rate of 0.5 μg/kg/min. for 8 hours. Thereafter a final sample of the liver was taken.

Blood samples were withdrawn from a catheter inserted into the femoral artery after induction of anesthesia, but before the liver biopsy and then at different time intervals during the infusion, as indicated in the figures.

The dogs were divided into two groups. Three dogs received only norepinephrine and three norepinephrine and nicotinic acid. Nicotinic acid was given rapidly intravenously as a 20 % solution. Immediately before the infusion was started 0.20 g/kg of nicotinic acid was given. Then 0.15 g/kg was given every second hour as indicated in the figures.

During the whole procedure blood pressure was measured with an Elema-Schönander pressure transducer from a tephlon catheter inserted into the femoral artery The pressure



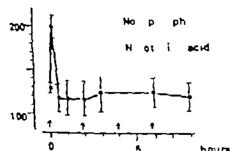


Fig. 2. Mean arterial pressure in the same groups of dogs as in fig. 1 The vertical bars indicate the standard error of the mean.

transducer was filled with saline. In the figures the pressure is given as the mean recorded pressure.

Lipid analysis

This was performed as described previously (2) It should be mentioned that nicotinic acid does not influence the titration of FFA.

Results

Effect on free fatty acids (FFA)

The results are given in fig 1 Norepinephrine caused an increase of FFA from 0.6 mEq/L to about 2 mEq/L during the infusion period. After nicotinic acid administration, however the FFA level was not significantly changed by the norepinephrine infusion until after 6 hours when a significant increase occurred ($P < 0.05$) There was a significant difference between norepinephrine infusion only and norepinephrine + nicotinic acid ($P < 0.01$) at all times except at 8 hours.

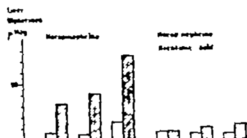


Fig 3. Liver glyceride concentration before and after infusion of norepinephrine for 8 hours in the same dogs as in fig 1
□ Before
▨ After

Effect on blood pressure

Fig 2 shows the blood pressure response to norepinephrine in the two groups. The mean pressure immediately after infusion was 202 ± 23 and 199 ± 13 mm of Hg respectively in the groups without and with nicotinic acid There was no significant difference in blood pressure during the entire infusion between the two groups.

Effect on liver glycerides

The infusion of norepinephrine caused a heavy increase of the liver glycerides as is seen in fig 3 The animals treated with nicotinic acid showed only a small increase. The norepinephrine group increased on an average from 9 to 49 μM/g liver while the other group increased from 6 to 11 μM/g liver

Discussion

It has previously been demonstrated in dogs that nicotinic acid blocks the FFA release induced by short time infusion of norepinephrine while the pressor

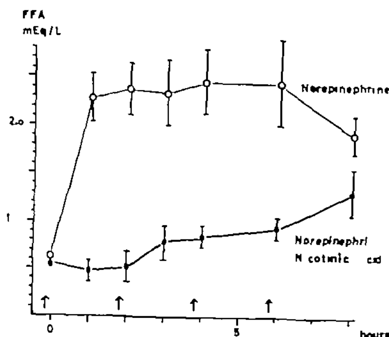


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effect remains unchanged (3) Our results here show that also over long periods of infusion of norepinephrine, nicotinic acid in the dosage used, significantly diminished the rise in FFA up to 8 hours. At the same time there was no difference in blood pressure between the two groups

The treatment with nicotinic acid almost completely prevented the fatty infiltration of the liver These findings lend additional support to the theory that catecholamines induce fatty liver by mobilising FFA from adipose tissue to the liver Furthermore in our experiments any eventual secondary effects of catecholamines contributing to the accumulation of lipids in the liver such as circulatory changes, have been ruled out.

It is worthwhile to mention that the plasma glycerides which are derived almost exclusively from the liver showed a small decrease in both groups during the experiment. However since the liver glyceride pool is about ten times the plasma glyceride pool, these small changes in plasma glyceride concentration are irrelevant with regard to the changes in the liver unless there were major changes in turnover rates.

Our results have also indicated the possibility of preventing fatty infiltration of the liver when this is related to a catecholamine induced flux of FFA from adipose tissue to the liver

Summary

The concentration of plasma free fatty acids (FFA) the blood pressure and the liver glyceride concentration has been studied in two groups of dogs. The first group was given a continuous infusion of norepinephrine and the second obtained in addition nicotinic acid which blocks the catecholamine induced stimulation of FFA mobilisation from adipose tissue.

The mean blood pressure did not differ between the two groups. FFA increased about 4 times above the basal level in the first group and this increase was almost completely blocked in the second The liver glycerides increased about 5 times in the first group and this increase was considerably reduced after nicotinic acid treatment The findings have been discussed with regard to the relationship between increased FFA levels and the development of fatty liver

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SUBJECT INDEX

Arteries

Asthma

Blood

Leitfaden der Gastroscopie Gastrophotographie und Magenbiopsie By W Brühl. 95 pp. Price D M. 19 80 Georg Thieme Verlag Stuttgart 1962

In 1951 the present author and H Kalk published their book Leitfaden der Laparoscopie und Gastroscopie. Laparoscopy has been excluded from the present account, which has meant that additional space could be devoted to gastroscopy and its latest developments gastrophotography and gastrobiopsy

Following a brief historical sketch the author discusses the indications for gastroscopy mentions the most usual forms of gastroscope and discusses their advantages and disadvantages. He then goes on to gastrophotography and refers to the following instruments for colour photography Wolf Schindler Mancke Sass Wolf Debray's instrument with electron flash equipment is mentioned only in passing For one who has had the opportunity of using this admirable instrument for several years, it would seem to merit a more prominent place in the discussion. The book contains 15 colour plates Remarkably however only three of them seem to be photographs, the remainder being ordinary drawings. One would have expected the entire illustrative material to consist of colour photographs. The chapter on gastrobiopsy is excellently handled Credit is rightly given to the great work done by Henning's clinic for the proper understanding of the normal picture of the mucous membranes and gastritis. The importance of the suction biopsy technique is stressed

The purely clinical part of the presentation is concerned with the gastroscopic picture in gastritis, gastric ulcer polyps and gastric carcinoma, and with findings in the operated stomach.

The presentation as a whole is concise and easy to read. In conclusion the book may be recommended for instruction in gastroenterology and to interested internists and surgeons.

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Congenital malformations A Ciba Foundation Symposium. Ed G E. W Wolstenholme. 308 pp 91 ill Price 45s. net. J & A. Churchill, London 1960

This volume records the proceedings of the January 1960 Ciba Conference on Teratology held under the chairmanship of the well-known Professor of embryology W J Hamilton. Top scientists give excellent summaries of their work associated with teratogenesis in its broadest sense, including chromosomal abnormalities (C. E. Ford) the effect of environmental factors (T H. Ingalls) antimetabolites (M L. Murphy) and PGA deficiency (Marjorie M. Nelson) There are valuable reviews of the morphogenesis of anencephaly (A. Giroud) and hydramnios associated with congenital malformations (A. C. Stevenson) All papers are accompanied by the discussions and the book concludes with a 24-page general discussion in which attention is also paid to the placenta The Ciba Symposium is a most valuable book of reference and a good summary of the recent views on teratogenesis, experimental and human

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